# IMSERC User Manual for NMR

## CONTENTS

## INTRODUCTION

This manual is intended to give you instructions on how to setup the routine NMR experiments with Topspin and IconNMR interfaces. Use of this instrument is allowed only by qualified users after receiving training by a staff member. Do not run this instrument without approval from IMSERC staff. Failure to do so may cause damage to the instrument, produce invalid data, and result in additional fees and/or removal of all IMSERC privileges. This short set of instructions is meant to serve as a guide for 'routine' data collection on the instrument. For custom experiments, contact a staff member. Please read this standard operating procedure and acquaint yourself with the instrument. If during the course of using the system, something happens that you do not understand, please ***stop*** and ***get help***. In any event, be completely prepared to justify your actions. The cost of even minor repairs is considerable.

## SAFETY

All users of IMSERC must review the general safety policies at http://imserc.northwestern.edu/about-policies.html. To become an independent user of this instrument, you must have the following safety training and certificates that are offered at https://learn.northwestern.edu:

- Laboratory Safety
- Strong magnetic field safety
- Personal Protective Equipment

You need the above certificates in order to be able to reserve time for this instrument on NUCore. Upon completion of the certificate, it will take an overnight to filter through the different systems and get into the files that NUCore uses. Additionally, familiarize yourself with the location of standard safety stations like eyewash and shower stations found in the west side of the NMR room. Protective eyewear is required in this room, and gloves should be removed when using the computer.

## DATA MANAGEMENT

The following template is used to save your fids: /home/walkon/data/YourGroupFolder/YourNetid/SampleName. Your personal data folder is created during training. Please save data under your personal folder, which must be located under your group folder (supervisor's last name). See a staff member if you need help to create your personal folder on the instrument.

Data on the instrument is copied to 'imsercdata.northwestern.edu' in real time. You can access your NMR data through imercdata.northwestern.edu/YourGroupFolder/nmr/NMRInstrumentName. Please follow instructions at http://imserc.northwestern.edu/about-general-faq.html#data for details about data access.

## SOFTWARE

Offline data processing and analysis can be performed with MNOVA and Topspin. Northwestern has campus wide license for MestraNova. Please refer to Mnova installation instructions at http://imserc.northwestern.edu/about-general-faq.html#software to install the program on your computer. Make sure to connect to Northwestern VPN when you work off campus. You are also encouraged to download the Topspin software from Bruker at https://www.bruker.com/service/support-upgrades/software-downloads/nmr.html and claim your free academic license.

## DEFAULT INSTRUMENT STATUS

The default interface on Ag500 and X500 is **IconNMR**. Please do not change! You do not need go through NUCore to use these two fully automated NMR instruments.

The default interface on A600, Au400, and HFCN600 is **Topspin**. Computer screen is by default deactivated. You must start your reservation through NUCore in order to turn on the computer screen. You have to login to NUCore to start your reservation before you can use. You can optionally start IconNMR if you want. Please return the instrument back to Topspin after you finish using IconNMR. Please end your reservation on NUCore after you finish.

The Hg400 (solids) is always on **Topspin**. Similar to A600/Au400/HFCN600, you have to go through NUCore to start/end your reservations. It has two probes, 4mm HX and 1.6 mm HFX. Notify the staff member in advance, if you need use a different probe than the one is currently installed.

If there is an error or problem with the instrument, please report the issue by following at least one of the steps below:

1. If you have already started your reservation using NUCore, please logoff by selecting the error reporting option and a brief description about the issue

2. If you have not started your reservation using NUCore, please report problems with the instrument at http://imserc.northwestern.edu/contact-issue.html add place the 'Stop' sign near the instrument computer. 'Stop' signs are located next to instrument and online at the link above
3. Email or talk to a staff member

## SAMPLE PREPARATION

Please use clean, non-scratched 5mm NMR tubes to prepare your samples. Some reputable NMR tubes vendors are listed here: http://imserc.northwestern.edu/nmr-links.html

The sample volume should be between 0.5 to 0.7 ml, preferably 0.55 ml for best shimming results. The solution should be clear of precipitation or suspended particles by going through filtration or centrifugation.

## FIVE STEPS TO USE IMSERC NMR

1. Login to NUCORE with your netid and logon to the instrument:
   a) turn on the computer monitor at instrument,
   b) your usage count starts
2. Login to instrument with your operator id (usually same as netid)
3. Load your sample and run your experiment
4. Logout from the instrument
5. Login to NUCORE and logout your instrument session:
   a) turn off the computer monitor at instrument,
   b) your usage count stops

## COMMON COMMANDS/PARAMETERS IN TOPSPIN

### SETTING UP EXPERIMENTS & PROCESSING

| | |
|---|---|
| sx 10 | to put #10 (could be any position) sample on the autosampler into magnet. |
| sx ej | to put sample inside magnet back to the autosampler |
| rga | automatically set receiver gain |
| zg | start acquisition |
| tr | transfer data (while acquisition is in progress) |
| multizg | start multiple acquisitions starting from current dataset |
| go | submit experiment to acquisition |
| stop | abort an acquisition, losing all the FID data recorded so far |
| halt | halt the running acquisition, saving the recorded FID data to hard disk |
| efp | weighted Fourier Transformation for 1D dataset |
| apk | do automatic phase correction |
| abs | automatically optimize baseline |

xfb weighted Fourier Transformation for 2D dataset

**IMPORTANT PARAMETERS FOR ACQUISITION**

P1 F1 channel 90° pulse width, micro seconds

P2 F1 channel 180° pulse width

RG Receiver gain

D1 relaxation delay, 1 to 5 times T1

2TD Time domain data points for F2 (direct dimension)

2SW spectral width in ppm for F2 (direct dimension)

1TD Time domain data points for F1 (indirect dimension)

1SW spectral width in ppm for F1 (indirect dimension)

AQ Acquisition time in seconds

NS Number of scans

DS Number of dummy scans

NUC1 – NUC8 Nucleus observed (1H, 13C, 31P, 19F, etc.)

O1 – O8 Frequency offset for channel 1 – 8 in Hz

O1P – O8P Freq. offset for channels 1 – 8 in ppm

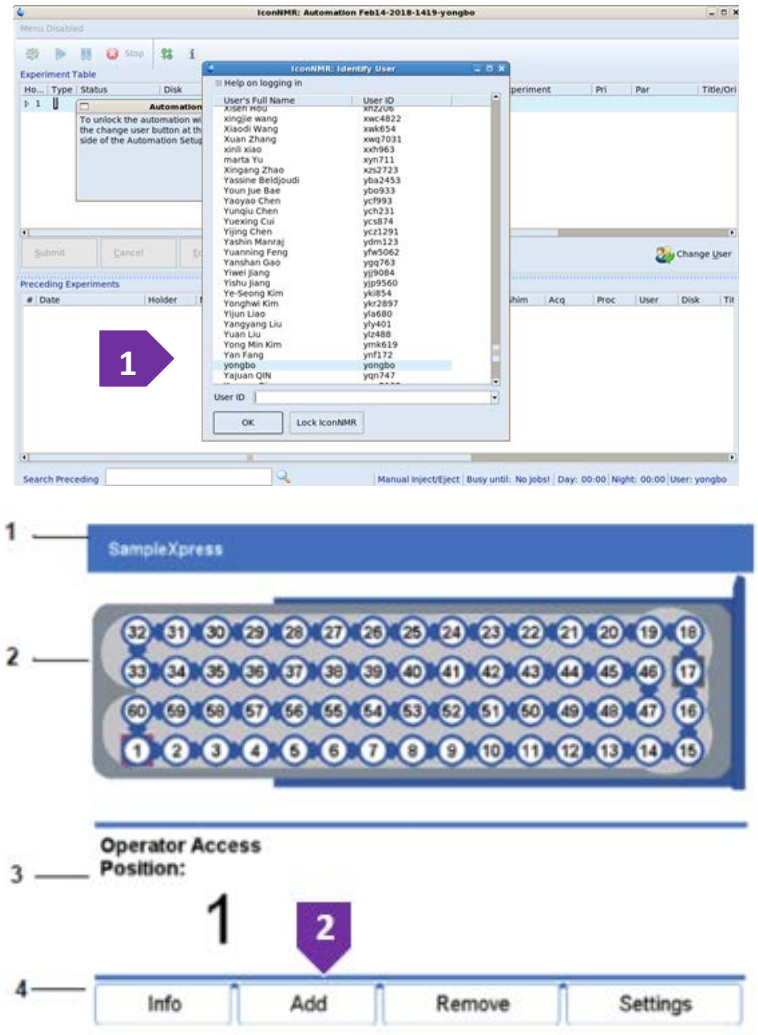
SFO1 – SFO8 Freq. for channels 1 – 8 in MHz

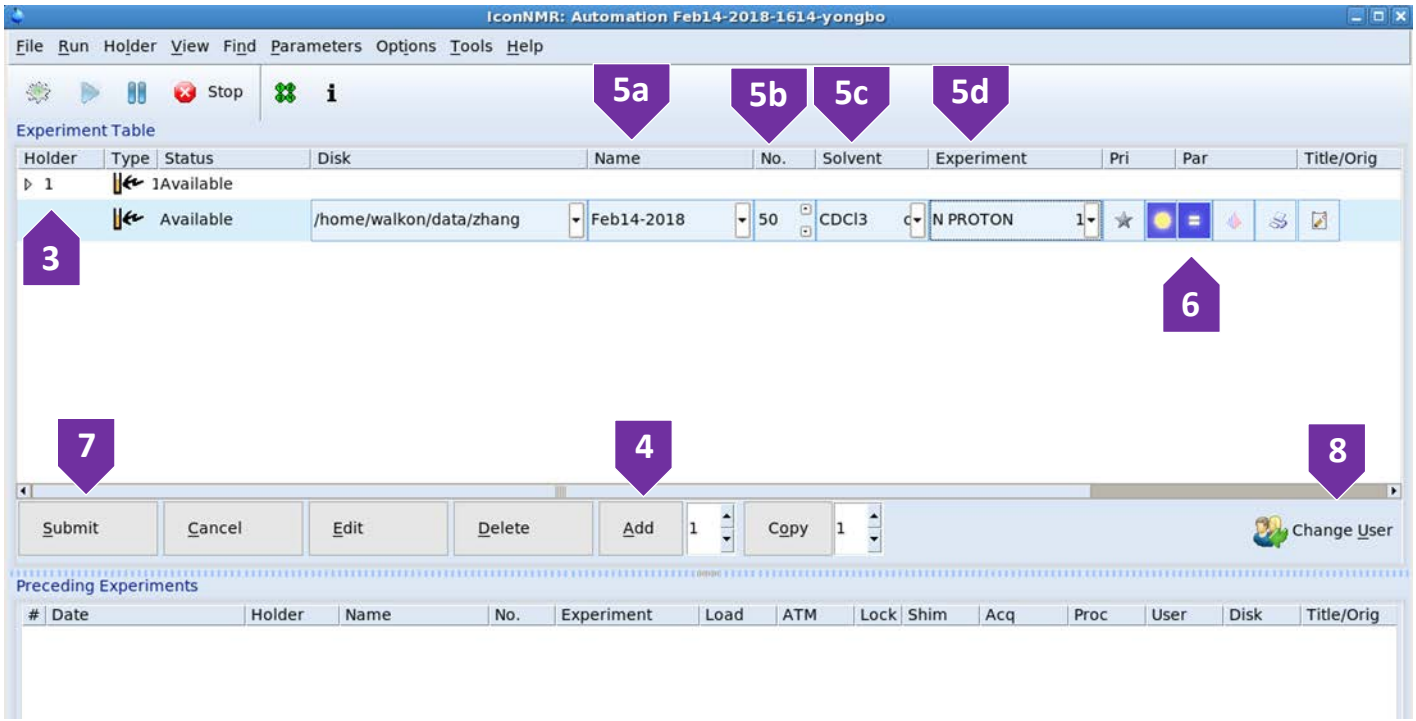
## DATA ACQUISITION:

### USE ICONNMR W/ SAMPLEXPRESS

1. Login with your operator ID
2. Load your sample to SampleExpress
3. Click the Holder # where you sample is loaded.
4. Click Add
5. Fill in following fields: Name (5a), No. (5b), Solvent (5c), and Experiment (5d)
6. Change parameters if needed
7. Click Submit
8. Logout ICON by clicking on “change user”

**Fig 4**: Steps to setup NMR experiment with IconNMR





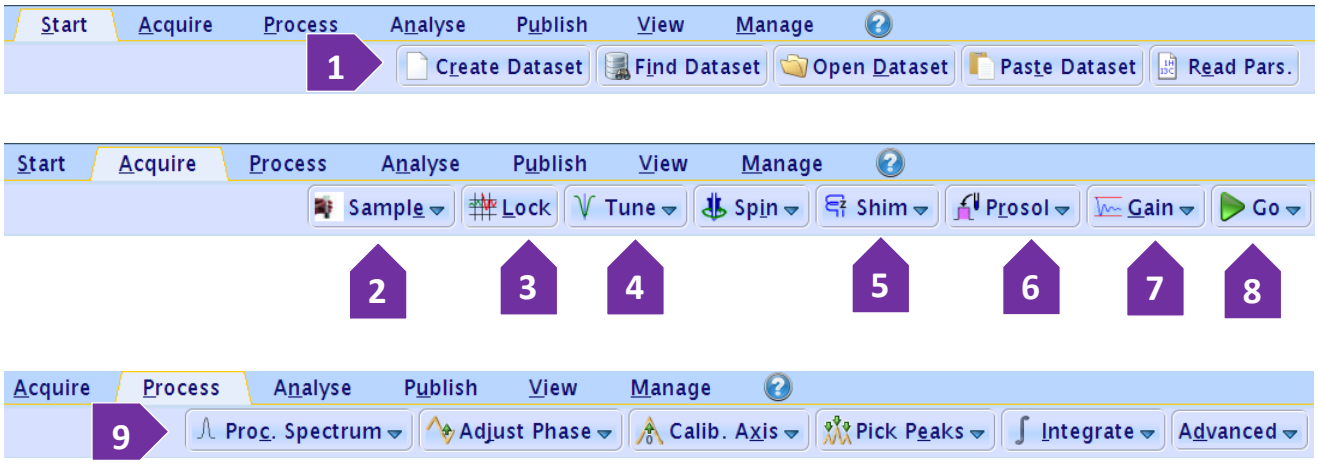

Notes for middle panel of fig 4 (step #2, labeled with 1-4 in black):

1. Message display
2. Displays the cassette with its 60 positions.
   - Gray positions are empty positions.
   - White positions are loaded with samples.
3. Information about Operator Access Position and Sample inside magnet.
4. Touch screen buttons to **Add** and **Remove** samples.

**INTERACTIVELY SETUP EXPERIMENT WITH TOPSPIN**

1. Create new dataset and setup initial parameters
2. Insert/Eject Sample
3. Lock on your selected Solvent
4. Tune/Match the Probe to the nuclei of your experiment
5. Shim
6. Load probe related parameters
7. Auto set receiver gain
8. Acquire fid
9. Process data

**Fig 1**: Flow chart for interactively setting up NMR experiment with TOPSPIN




**Parameters setting for 1D and 2D experiments**

The arrowed fields at **Fig 2** (1-5) need to be filled and changed at **Fig 3** (1-8) based on your needs.

**Input to the "new dataset" window:**

1. Sample name
2. Experiment number
3. Experiment to run
4. Solvent used
5. Directory for the dataset (your folder under your group name)
6. Change the parameters for fields 1-8 at Fig. 3 in next page based on your needs

**Fig 2**: Input to the "new dataset" window

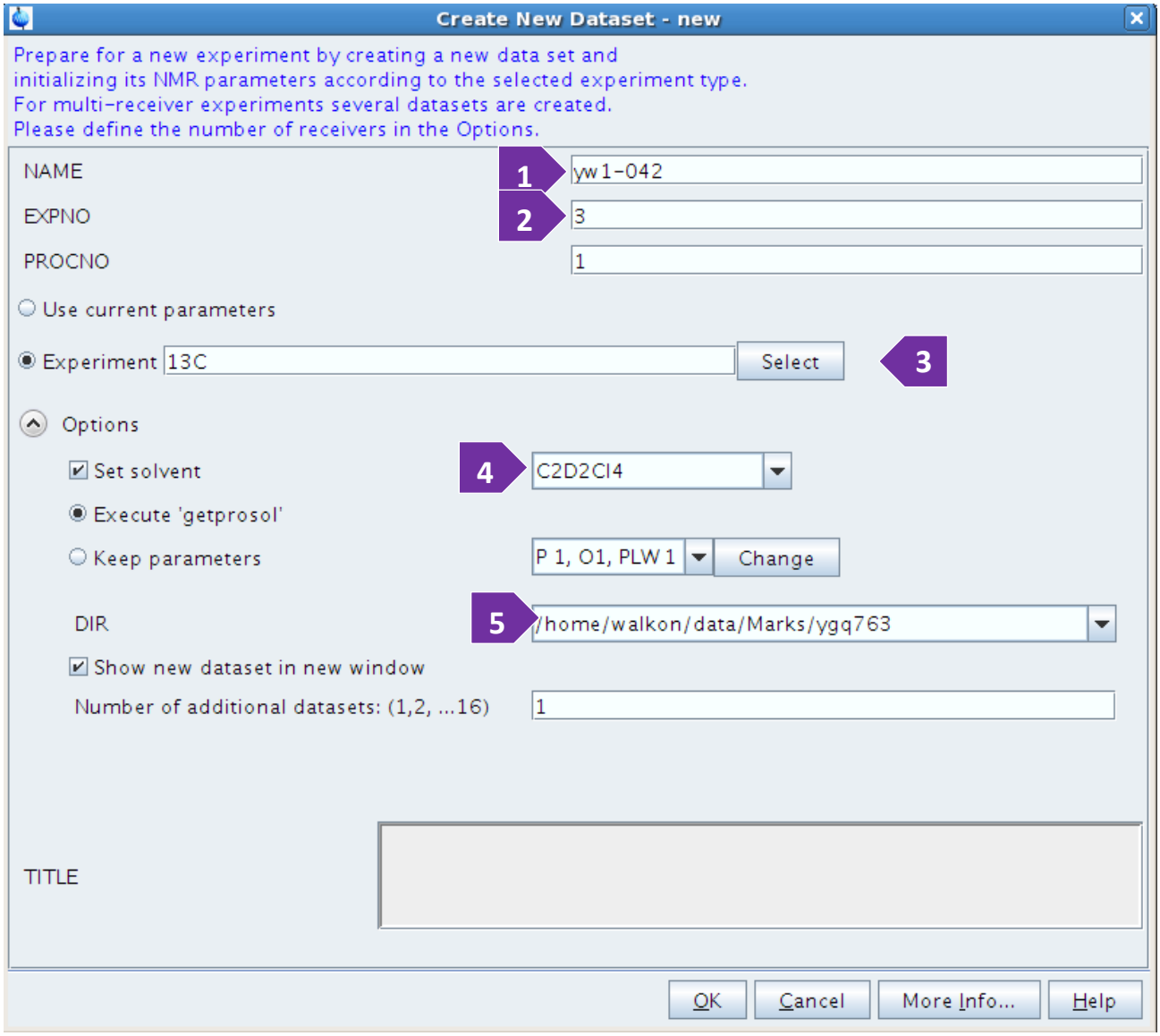
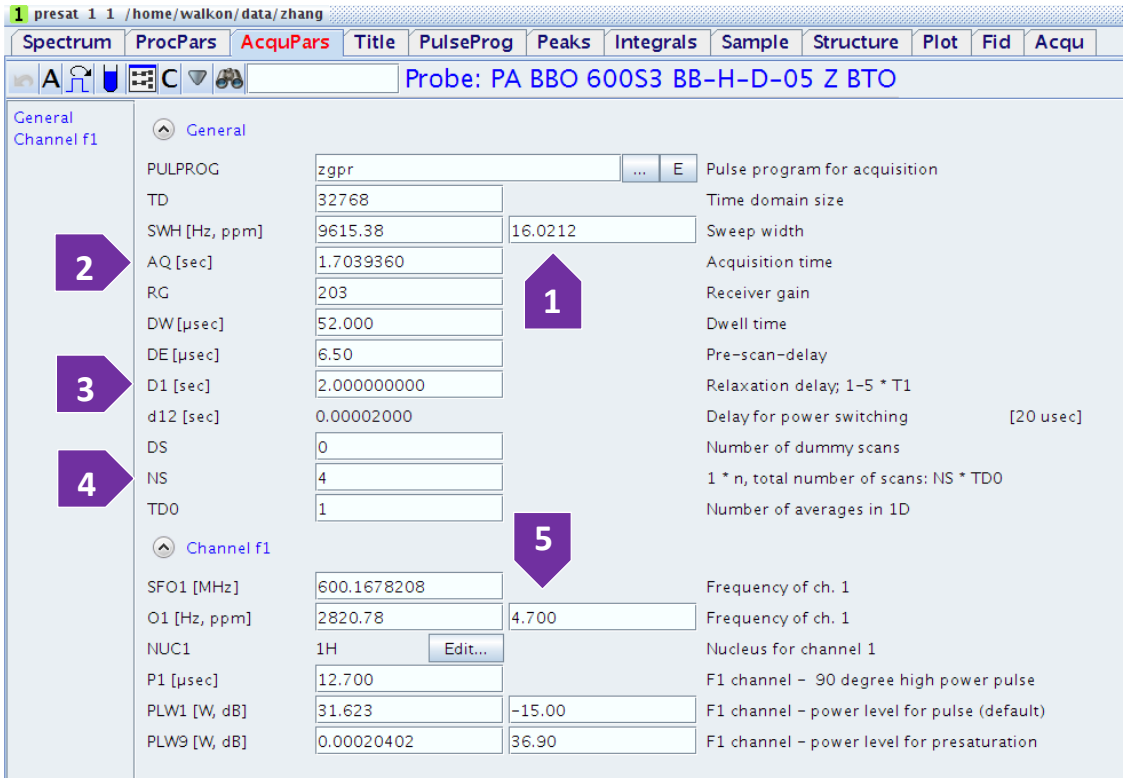
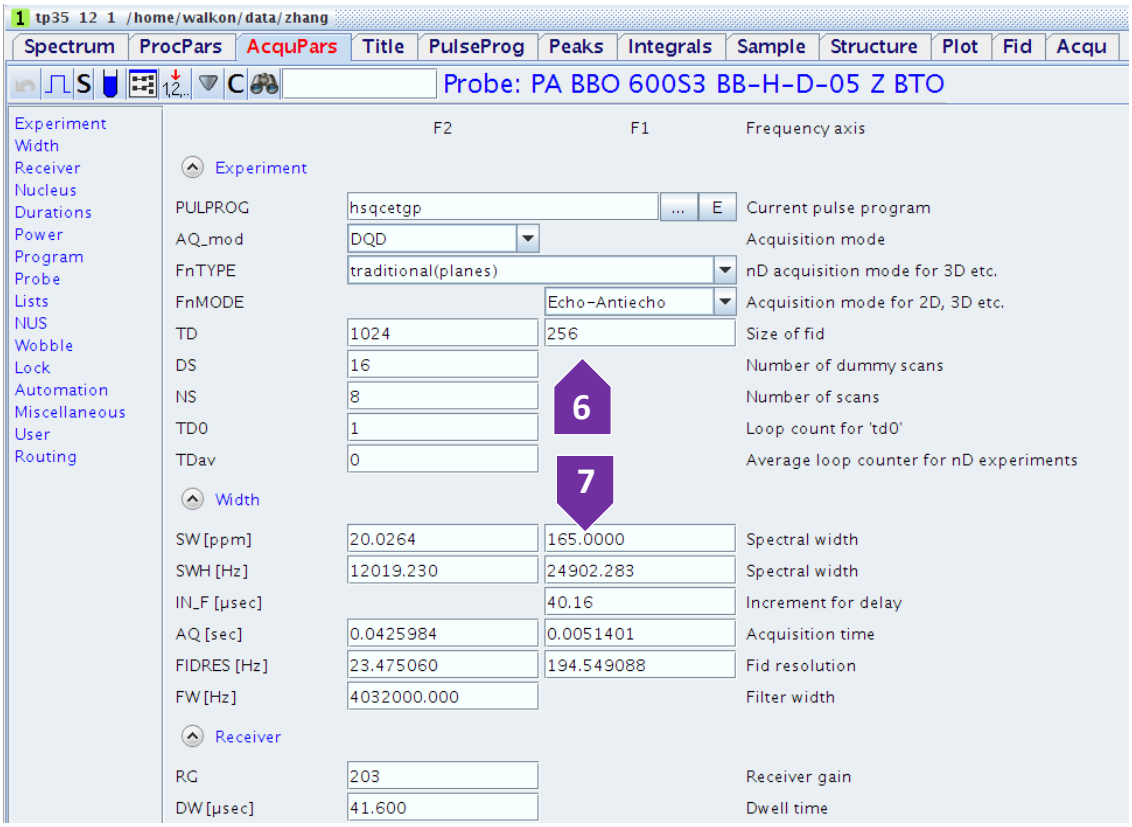
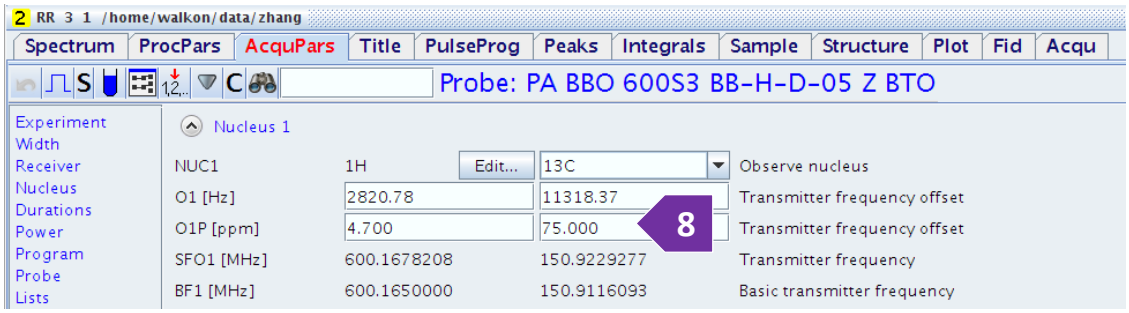


**Fig 3**: Important parameters might need to be changed




2 RR 3 1 /home/walkon/data/zhang
Spectrum
ProcPars
AcquPars
Title
PulseProg
Peaks
Integrals
Sample
Structure
Plot
Fid
Acqu
Probe: PA BBO 600S3 BB-H-D-05 Z BTO
Experiment
Width
Receiver
Nucleus
Durations
Power
Program
Probe
Lists
Nucleus 1
NUC1
1H
Edit...
13C
Observe nucleus
O1 [Hz]
2820.78
11318.37
Transmitter frequency offset
O1P [ppm]
4.700
75.000
Transmitter frequency offset
SFO1 [MHz]
600.1678208
150.9229277
Transmitter frequency
BF1 [MHz]
600.1650000
150.9116093
Basic transmitter frequency
8

## DATA ANALYSIS

MNOVA has an extremely comprehensive manual accessed through "help". A simplified MNOVA manual can be found at http://imserc.northwestern.edu/downloads/nmr-mnova_chemists8_simplified.pdf. The most useful Topspin manuals for processing is at http://imserc.northwestern.edu/downloads/nmr-processing-reference.pdf

## PUBLICATION

### EXPERIMENTAL SECTION

Write a paragraph similar to the 'experimental section' found in a publication. Details about every instrument component and methods must be provided in this paragraph. The NMR spectrometer specifications can be found in the table at "**ACKNOWLEDGEMENT**" section below. You need only the content in the parenthesis. For example, you should write "Bruker NOE 600 MHz spectrometer equipped with QCI-F cryoprobe" if you use HFCN600, DO NOT write "HFCN600" as the name of NMR spectrometer in your publication.

The important information about your NMR experiment includes but not limited to, the experiment name, spectral width, acquisition time/time domain points, window function for apodization etc.

### ACKNOWLEDGEMENT

Acknowledgement info is listed under http://imserc.northwestern.edu/about-acknowledgements.html.

If your work used the HFCN600 please use this acknowledgement:

This work made use of the IMSERC at Northwestern University, which has received support from the NIH (1S10OD012016-01 / 1S10RR019071-01A1), Soft and Hybrid Nanotechnology Experimental (SHyNE) Resource (NSF ECCS-1542205), the State of Illinois, and the International Institute for Nanotechnology (IIN).

For other NMR instruments, please use this acknowledgement:

For This work made use of the IMSERC at Northwestern University, which has received support from the Soft and Hybrid Nanotechnology Experimental (SHyNE) Resource (NSF ECCS-1542205), the State of Illinois, and the International Institute for Nanotechnology (IIN).

| Instrument | Funding Source |
|---|---|
| HFCN600 (Bruker NOE 600 MHz w/ QCI-F cryoprobe) | NIH 1S10OD012016-01 / 1S10RR019071-01A1 |
| A600 (Bruker AVANCE III 600 MHz w/ BBFO probe) | NU / Int. Institute of Nanotechnology |
| Ag500 (Bruker AVANCE III 500 MHz w/ DCH cryoprobe) | NU |
| X500 (Bruker AVANCE HD 500 MHz w/ Prodigy probe) | Int. Institute of Nanotechnology |
| Hg400 (Bruker AVANCE HD 400 MHz w/ MAS solids probe) | Int. Institute of Nanotechnology |
| Au400 (Bruker AVANCE HD Nanobay 400 MHz w/ BBFO probe) | NSF CHE-1048773 |

## TROUBLESHOOTING

1. If you need go to Topspin interface by exiting IconNMR on A600, Au400, and HFCN600, please login with username “walkon” (with password “GO**********”), stop automation first, then quit IconNMR.
2. If you have to reboot the computer for NMR instrument, login with account “walkon” (with password “GO******). Click the “Topspin” icon on desktop to start acquisition software. Leave the acquisition software open when you are done with the measurement.
3. At this time, if you experience difficulty with SampleXpress autosampler, please contact a Staff.

## APPENDIX A: LIST OF COMMON EXPERIMENTS ON ICONNMR

| Experiment entries | Description | Recommended setting for ~10mg material, please modify "NS" accordingly based on your sample concentration |
|---|---|---|
| **PROTON_icon** | routine 1D proton spectrum | **Common parameters for all experiments:**<br><br>**D1**: relaxation delay, 1 to 5 times of T1, 2-5 seconds<br>**SW**: spectral width in ppm for F2 (direct dimension)<br>**AQ**: Acquisition time in seconds<br>**NS**: number of scans<br>**O1P**: offset freq for channels 1 in ppm, usually 1H<br>**O2P**: offset for channel 2, usually 13C<br>**1TD**: Time domain data points for F1 (indirect dimension), aka number of increments<br>**1SW:** spectral width in ppm for F1 |
| **C13CPD_icon** | 1D $^{1}$H-decoupled $^{13}$C spectrum | |
| **C13DEPTQ135_icon** | DEPTQ 135 experiment detect all Carbons - CH3/CH positive CH2/C negative | |
| **C13IG_icon** | 13C with inverse gated 1H decoupling no NOE for quantitative NMR | |
| **C13DEPT90_icon** | DEPT 90 experiment only CH | |
| **C13DEPT135_icon** | DEPT 135 experiment CH3/CH positive CH2 negative -$^{13}$C 1-bond correlations, all peaks positive (dept-45 analog) DEPT-135 experiment | |
| **gCOSY_icon** | Gradient selected COSY | |
| **COSYDQF_icon** | COSY with double quantum filter | |
| **HSQC_EDIT_icon** | 1H-13C multiplicity edited HSQC with gradient selection | |
| **HSQC_icon** | 1H-13C 1-bond correlations, all peaks positive, HSQC with gradient selection | |
| **HSQC_EDIT_NUS_icon** | 1H-13C multiplicity edited HSQC with gradient selection Non Uniform Sampling w/ 25% sampling density | |
| **HMBC_icon** | 1H-13C HMBC with gradient selection using 3-fold low pass filter for better 1J suppression | CNST13 = 3-12 Hz (default 8Hz for $J_{2/3}$CH) |
| **HMBC_NUS_icon** | 1H-13C HMBC with gradient selection using 3-fold low pass filter for better 1J suppression Non Uniform Sampling w/ 50% sampling density | |
| **TOCSY_icon** | Phase sensitive 2D TOCSY experiment using MLEV-17 mixing | d9 = 30 to 120 ms |
| **NOESY_icon** | Phase sensitive NOESY 1H-1H correlations based on proximity also for exchange | d8 = 0.1 to 1 second |
| **ROESY_icon** | 1H-1H correlations based on proximity for intermediate MW around 1600 Da | p15 = 0.1 to 0.5 second |
| **WATER_SUPP_icon** | Solvent suppression with noesygppr1d sequence | |

## APPENDIX B: VARIABLE TEMPERATURE CONTROL FOR NMR

### INTRODUCTION

This manual is to instruct NMR IMSERC users to do the Variable Temperature (VT) experiments on A600, Au400, and Hg400 (w/ RT probes) NMR spectrometers (-150 to +150 °C). In principle, one can follow similar procedure to use the HFCN600 (equipped with QCI-F cryoprobe, with much smaller VT range from -30 to 70 °C).

Depending on your target temperature, please consult with **fig 1** and **table 1, 2** to pick the right spinner, set up the gas flow rate, and the cooling power for the BCU II device.

When operating at elevated temperatures, internal probe heating is sufficient and precisely regulated with the new BSVT on Au400 and Hg400, which also controls and regulates the selected VT flow rates and other auxiliary flows. Chillers are not required as long as the operating temperature is sufficiently above the room temperature. For A600, you need manually adjustments of Shim/Flush gas. If you need temperatures below room temperature, chiller (BCU II or FTS) is required.

Always remember to redo the lock, tune, and shimming if the temperature changes more than 10 °C. The solvent boiling and/or frozen point must be considered. For high temperature, the highest temperature allowed should be at least 10 °C below the boiling point while for a low temperature experiment; the lowest temperature should be at least 5 °C above frozen point.

The real temperature and detected temperature may be slight different. Please refer to the temperature calibration curve for correction or calibrate temperature yourself. If you need a temperature below -60 °C, please ask NMR staff to use liquid nitrogen as a cooling source.

### OPERATION PROCEDURE

1. Select the right spinner based on **fig 1** and **table 1,** position and load your sample.
2. Open temperature control panel by typing command **edte**.
3. To change gas flow rate, click "**set**" under "Target Gas Flow" on BCU II (**fig 2**), put appropriate gas flow rate in the popup box. For FTS chiller, simple click on "**-**" or "**+**" to change the flow rate (**fig 3**).

4. To change the chiller power, for FTS chiller, set the desired temperature using the ▲▼arrow. For BCU II, click "**set**" under "Target Power" and select cooling power based on **table 2**.
5. To change temperature, click "**set**" under "Target Temperature" on BCU II (**fig 2**) to set your target. For FTS, click "**change**" button of "Target temp" row. Change the temperate by 10 °C increment and give around 5 minutes before doing next increment. Changing temperature too fast may damage the probe.
6. For FTS chiller, you can explicitly set the temperature. The maximum you can set is 60 °C. Please keep FTS at least 10 °C below your target temperature for above RT experiment. For extremely low experiment, you have to keep the FTS temperature 30 to 40 °C below your target.
7. Once the target temperature is regulated, please wait at least 5-10 minutes for the sample to reach equilibrium before you run your experiment. Remember to redo locking, tuning, and shimming for each different temperature.
8. Collect data.
9. After finishing, please restore the temperature setting back to default at 25 °C. Change flow rate and cooling power back to default.

**Fig 1:** Three types of spinners: Standard **POM** Spinner (blue, left), 0°C to +80°C; **Kel-F** Spinner (off white, middle) for elevated Temperatures, +80°C to +120°C; **Ceramics** Spinner (white, right) for high and low Temperatures, +120°C to +180°C and 0°C to -150°C (A600, Au400, and Hg400)

**Table 1:** The VT gas, shim gas, flush gas settings and recommended spinners for all 5 mm RT probes (P = POM ; K = Kel-F; C = Ceramics):

| Sample T [°C] | -150…-80 | -80…-50 | -50…0 | 0...80 | 80...120 | 120...150 |
|---|---|---|---|---|---|---|
| Spinner | C | C | K & C | P, K & C | K & C | C |
| Recom. VT gas [L/hour] | 1200 | 1000 | 750 | 400 (P) 600 (K & C) | 450 | 350 |
| Shim gas [l/min] | 20 | 20 | 20 | 0 | 0…20 | 20…60 |
| Flush gas [l/min] | 5…10 | 5 | 5 | 0 | 5 | 5 |
| Chiller | LN2 | LN2 | BCU II (-50) | | | |

**IMPORTANT NOTES (INSTUMENT DAMAGE WILL OCCUR IF NOT FOLLOWED):**

1. The temperature of the shim system should always be -80 °C < T < 80 °C.
2. The temperature of the magnet flange (O-Ring!) has to be 3 ºC < T < 80 ºC, especially for long term experiments.
3. The shim system and the probe has to be flushed during low temperature experiments (sample temperature T < 0 °C) to prevent icing and condensed water.
4. The new BBFO smart probes on Au400 and Hg400 automatically adjust the Shim/Flush gas based on temperature setting. The A600 needs manually adjustments of Shim/Flush gas to 20/10 from 10/6 LPM (ask NMR staff if you do not know how).
5. You should raise/lower the temperate incrementally by 10 °C for about every 5 minutes
6. Never turn VT gas off.

**Table 2**: BCU II cooling mode (off, low, medium, and strong)

| | |
|---|---|
| 25 °C to 150 °C | off |
| 25°C down to 0°C | low |
| 0°C to -20°C | medium |
| -20°C to -50°C | strong |

**Fig 2**: Au400/Hg400 VT interface with BCU II (by type command “edte)

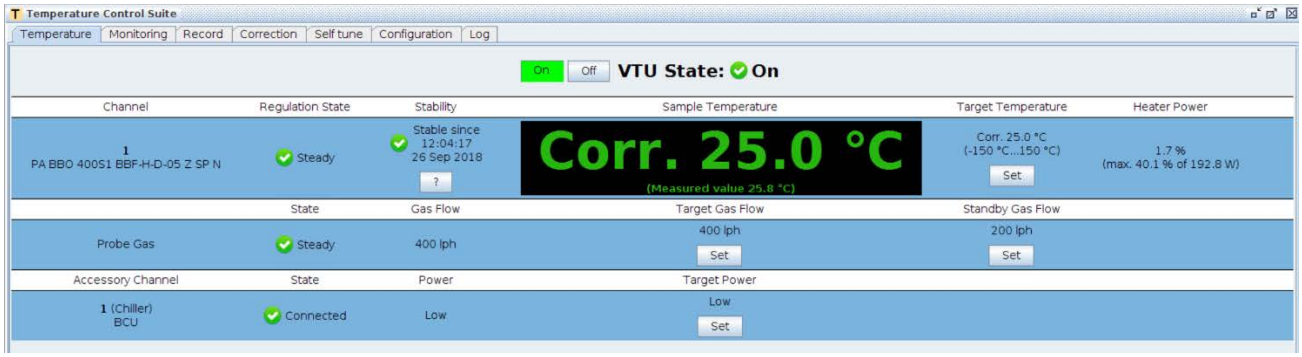
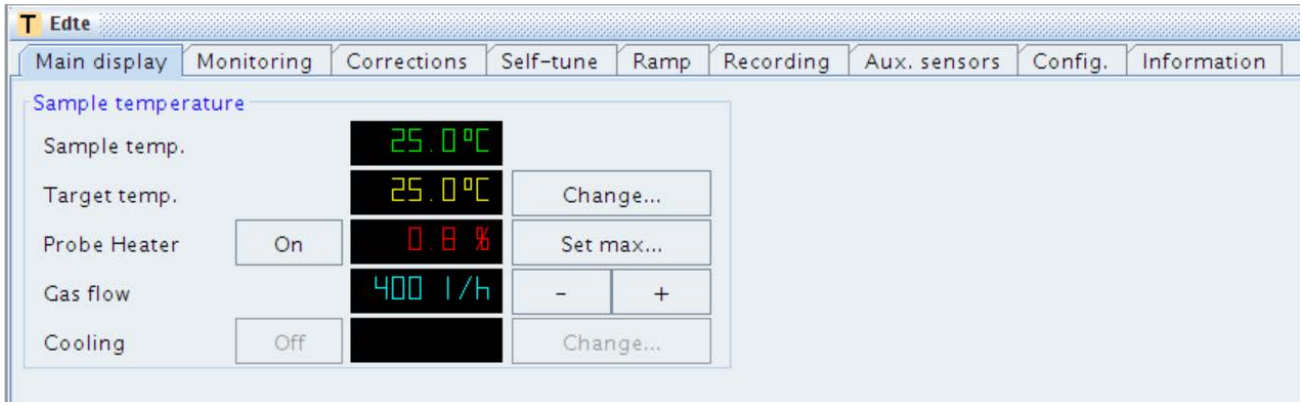



**Fig 3**: A600 VT interface with FTS (by type command “edte)



## APPENDIX C: 1D SELECTIVE NOESY WITH TOPSPIN

### REFERENCE SPECTRUM

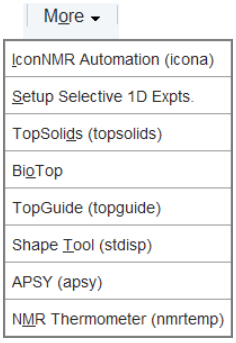
Run a 1D Proton spectrum, following the instructions in the short Bruker manual.

### SET UP THE 1D SELECTIVE NOESY

The selective pulse regions are set up using the integration tools. Power and duration of the shape pulses are calculated using the hard 90° pulse in the prosol table.

1. Stay in the reference spectrum, on the menu bar, click **Acquire**.

On the **More** button, click the **drop-down** arrow to see more options.

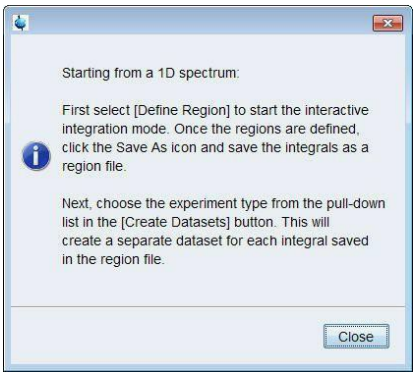



2. In the list, select **Setup Selective 1D Expts**.

The Workflow button bar changes for setting up the 1D selective experiment.

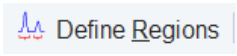
3. On the Workflow button bar, click **1D Selective Experiment Setup**.

4. In the message window, click **Close**.




There is no other function to this button then the instruction displayed above.

Expand the spectrum region having peaks you are interested in doing NOE.

5. On the Workflow button bar, click **Define Regions**. 


The Define Regions toolbar is displayed:


6. Integrate the peak (multiplet) that you will irradiate to observe the NOE.

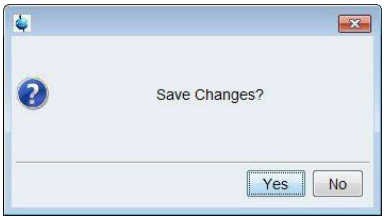
If desired, other peaks can be integrated and a separate dataset will be created for each integral saved in the region file.

7. On the toolbar, click **Save/export regions**  .

8. In the list, select **Save Regions to 'reg'**.

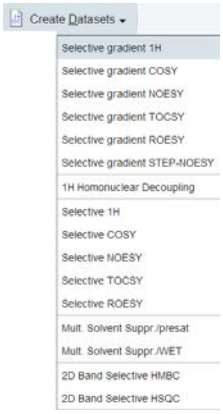
9. On the toolbar, click **Return do NOT save regions!**. 

10. In the message window, click **No**.



11. On the **Create Dataset** button, click the **drop-down** arrow to see more options.

12. In the list, select **Selective gradient NOESY**.



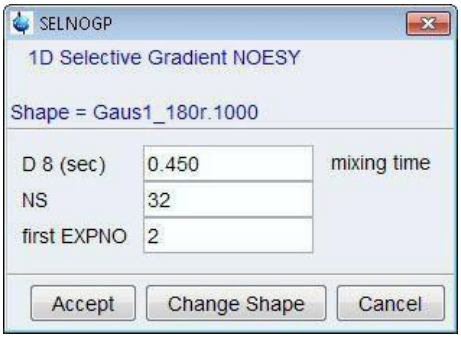

The default parameters are taken from the standard parameter set SELNOGP. The mixing time D8 is dependent on the size of the Molecule and the magnetic strength. It can vary from a large Molecule to a small one from 100 ms to 800 ms. If desired, the Gaus1_180r.1000 pulse can be changed by clicking on the Shape button in the above window. Number of scans (NS) should be determined based on your sample concentration.

13. Enter:

D8 = **0.450**

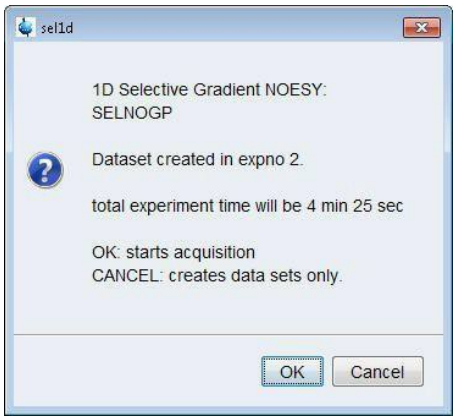
NS = **32**

14. In the SELNOGP window, click **Accept**.




The new dataset is created and all parameters are automatically set.

15. In the sel1d window, click **OK** to start the acquisition.




If you click “cancel”, dataset will be created but not run. You can make further changes and then start acquisition.

**DISPLAY 1D NOESY TOGETHER WITH 1D PROTON SPECTRUM**

1. Display the selective NOESY spectrum.

2. On the toolbar, click **Multiple display**.  The Multiple display toolbar is displayed:

3. Drag the Reference spectrum (1D proton) into the spectral window.

## APPENDIX D: PROTON DOSY EXPERIMENT

### INTRODUCTION

The **DOSY** (**D**iffusion-**O**rdered **S**pectroscop**y**) **experiment** provides accurate, noninvasive, molecular diffusion measurements on biofluids, complex chemical mixtures and multi component solutions. In DOSY spectra, chemical shift is along the detected F2 axis and diffusion coefficient is along the other F1 axis.

Molecules in the solution state move. This translational motion is known as Brownian molecular motion and is often simply called diffusion or self-diffusion. Molecular diffusion depends on a lot of physical parameters like size and shape of the molecule, temperature and viscosity.

Pulsed field gradient NMR spectroscopy can be used to measure translational diffusion. By use of a gradient pulse, molecules can be spatially labeled. After this encoding gradient pulse (δ), molecules move during the diffusion time (Δ). Their new position can be decoded by a second gradient pulse. This encoding/decoding procedure results

$$I(g) = I(o)\exp\left[-(\gamma g\delta)^2 D\left(\Delta - \frac{\delta}{3}\right)\right]$$

in an attenuation of the NMR signal which can be described by the following equation:

Where **I** is the observed intensity, **D** is the diffusion coefficient, **γ** is the gyro magnetic ratio of the encoded nucleus, **g** is the gradient strength, **δ** is the length of the gradient pulse, and **Δ** as mentioned previously is the diffusion time.

The diffusion experiment records a series of 1D $^{1}H$ spectra at increasing gradient strengths (g) and then fits the signal intensity decay to the above equation to obtain **D**.

Convection within the sample tube, such as, moving liquid columns along the sample axis (primarily due to temperature gradients), can seriously affect diffusion experiments, in particular, at elevated temperatures. Convection currents are caused by small temperature gradients in the sample and result in additional signal decay that can be mistaken for faster diffusion.

DOSY uses three parameters to define the duration of the diffusion: gradient length **δ** (**P30** in topspin, the total gradient defocusing time), the diffusion gradient level **g** (**GPZ6** in topspin, maximum 95%), and the diffusion delay

**Δ** (**D20** in topspin, 60 ms as default, max determined by the shortest T1 relaxation). In most case, **GPZ6** is the variable parameter to be arrayed for DOSY purpose. Depending on sample, you might need increase **D20** and/or **P30** (max **2ms**!) in order to obtain enough signal attenuation. The purpose of doing this is to get a diffusion decay curve like in the **figure C** below that will give you the best DOSY fitting.

There are two sets of parameter files under user directory:

**ledbpgp2s** ("longitudinal eddy current delay" LED-bipolar gradients pulse sequence)

**dstebpgp3s** (double stimulated echo for ***convection compensation*** and LED using bipolar gradient pulses for diffusion using 3 spoil gradients).

With LED, magnetization is stored along the z-axis during most of the pulse sequence, so T1 relaxation is predominant. Since in macromolecules the T1 relaxation is slower than the T2 relaxation, the LED experiment is better suited to the measurement of Ds of slower diffusing molecules where longer "diffusion delay" is required to detect attenuation of the signal.

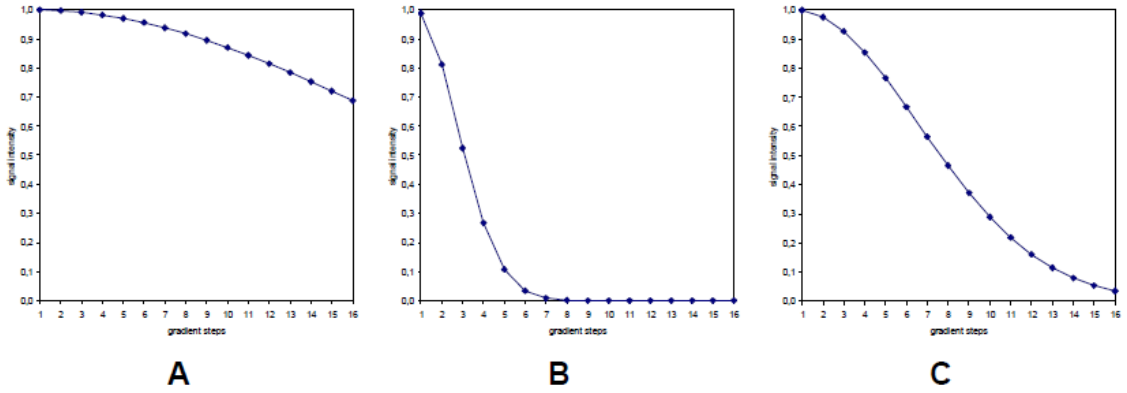



**Figure**: Simulated diffusion decay curves by varying the gradient strength **g** from 2 to 95% in 16 steps for the same diffusion constant, but with different selection for Δ and δ. They are chosen too small (A), too big (B), and properly (C) to sample data points along the whole decay curve.

**EXPERIMENT**

The DOSY pulse program used in the following procedure is the Stimulated spin-echo experiment using bipolar gradients and an additional delay just prior to detection for the ring-down of any possible eddy currents (**ledbpgp2s**). The same procedure works for **dstebpgp3s** if you need convection compensation.

1. To set up a DOSY experiment, start with recording a normal proton spectrum, followed by optimizing P1, SWH, and O1, if necessary.
2. Type "rpar ledbpgp2s1d_nu all" to retrieve 1D dosy parameters (or "rpar" to select "ledbpgp2s1d_nu"). Update solvent with yours (default is CdCl3)
3. Check to make sure the P1, SWH, and O1 are same as your proton experiment. The recycle delay D1 should be 1-2 T1 and dummy scan DS should be at least 8. Adjust NS accordingly to give sufficient S/N.
4. Change GPZ6 to 2% and type "zg" to collect data.
5. Use "edc" to create another 1D experiment and change GPZ6 to 75% and type "zg" to collect data
6. Click  (dual display) to compare the 1D data with GPZ6 at 75% to the previous 1D of 2% to check if the nmr signals of interest are attenuated to less than 5-10% of the intensities obtained with GPZ6 at 2%. If you don't get there or already past it, adjust GPZ6 (to 95% or 50%) accordingly to make sure you get there. Write down the GPZ6 value for 2D DOSY setup.
7. If changing GPZ6 alone is not enough to attenuate the signal enough, increase the D20 and/or P30 to achieve the goal.
8. Type "rpar ledbpgp2s_nu all" to retrieve 2D dosy parameters (or "rpar" to select "ledbpgp2s_nu"). Update solvent, P1, SWH, and O1 with the values from your proton experiment
9. Type" dosy" to create the gradient ramp function:
10. Enter first gradient amplitude: 2
    Enter final gradient amplitude: 95 (or the value obtained from 1D DOSY)
    Enter number of points: 16 (or the number you think appropriate for your sample)
    ram type (l/q): l
    and finally, Do you want to start acquisition? Select OK to collect 2D DOSY data.

**PROCESSING**

1. Set the proper window function.
2. Type "**eddosy**"
3. Type "**setdiffparm**" (or click  )
4. Type "**xf2**" (or click  )
5. If you need phase the spectrum, type "**rser 1**" to read the 1st fid to a new prono and type "**efp**" and "**apk**" to get correct **PHC0** and **PHC1** numbers. Then go back to 2D DOSY dataset and correct the phase values. Remember the phase mode is "**pk**" for direct dimension (F2).

6. Type "**dosy2d setup**" (or click  )
7. Type "**dosy2d**" (or click  ), you should see the 2D DOSY spectrum with chemical shift along the detected F2 axis and diffusion coefficient along F1 axis.

**ADDITIONAL NOTES**

Sample preparation: make sure the sample volume is not more than 550 ul.

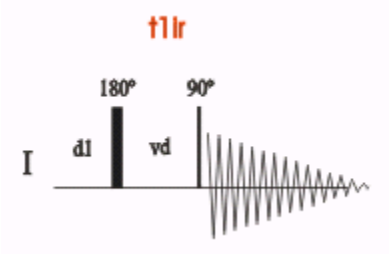
## APPENDIX E: NMR T1 RELAXATION EXPERIMENT

### INTRODUCTION

When an NMR sample sits in the magnet, the applied static magnetic field $B_0$ will generate the equilibrium magnetization $M_0$ along +z axis. When a RF pulse is allied to the sample, the net magnetization will be rotated away from +z axis. T1 relaxation (longitudinal or spin-lattice) is the process by which the net magnetization goes back to its initial maximum value ($M_{z,eq}$) parallel to $B_0$.

The inversion-recovery experiment measures $T_1$ relaxation times of any nucleus. If the net magnetization is placed along the -z axis, it will gradually return to its equilibrium position along the +z axis at a rate governed by T1. The equation governing this behavior as a function of the time t after its displacement is:

$$M_z(t) = M_{z,\mathrm{eq}}\left(1 - 2e^{-t/T_1}\right)$$




The basic pulse sequence consists of an $180^0$ pulse that inverts the magnetization to the -z axis. During the following delay, relaxation along the longitudinal plane takes place. Magnetization comes back to the original equilibrium z-magnetization. A $90^0$ pulse creates transverse magnetization. The experiment is repeated for a series of delay values taken from a variable delay list. A 1D spectrum is obtained for each value of vd and stored in a pseudo 2D dataset. The longer the recycle delay (d1) is, the more precise the T1 measurement is. Ideally d1 should be set to 5*T1. A rough estimation of the T1 value can be calculated from the null-point value by using T1=tnull/ln2.

### SETTING UP PROTON T1 EXPERIMENT

1. To set up a T1 experiment, start with recording a normal proton spectrum to adjust the spectral sweep width **SWH**, acquisition time **aq** and other parameter if necessary.
2. Create new dataset and load "**Proton_T1"** parameter set. Update the parameters with the ones you obtained from last step. The recycle delay **D1** should be ~2-5*T1. Adjust **NS** accordingly to give sufficient S/N (**fig 1**).
3. Edit the "t1delay" by clicking on 

at VDLIST line in **fig 1. Fig 2** is a good starting list.

4. Change the "TD" value for F1 dimension to the number in your VDLIST (**fig 3**)
5. Collect the pseudo 2D T1 dataset

**Fig 1**. ACQUPARS display in "pulse program parameters" view

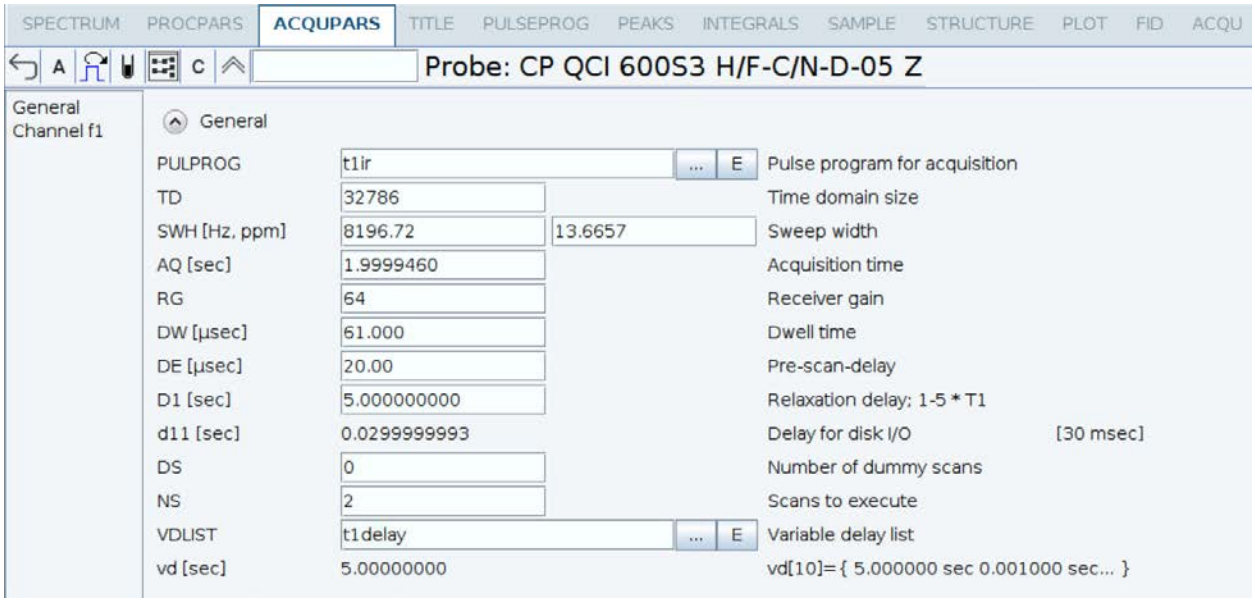
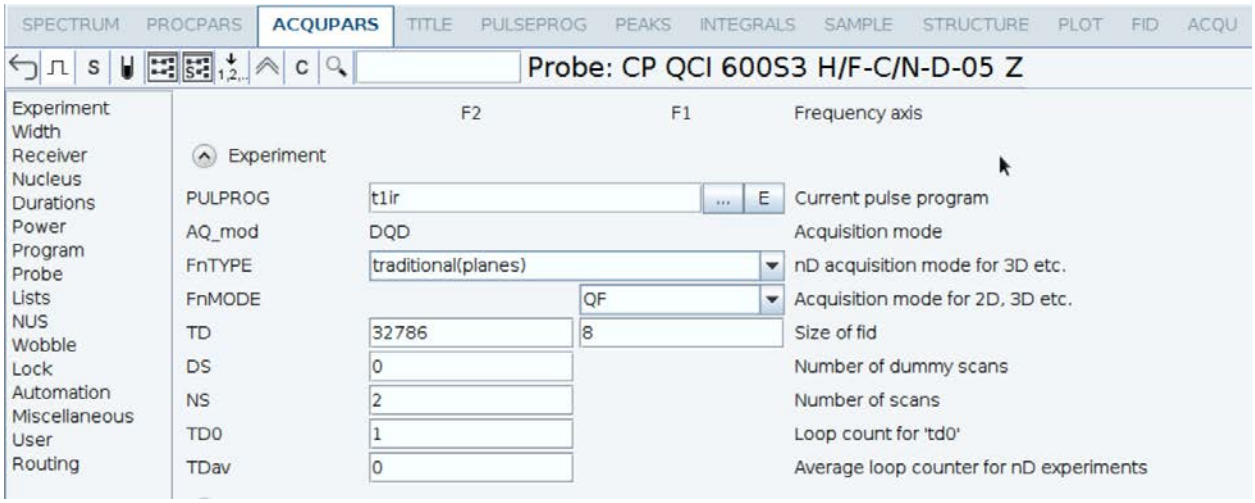



**Fig 2**. An example of t1delay list with 8 delays

**Fig 3**. ACQUPARS display in “all acquisition parameters” view

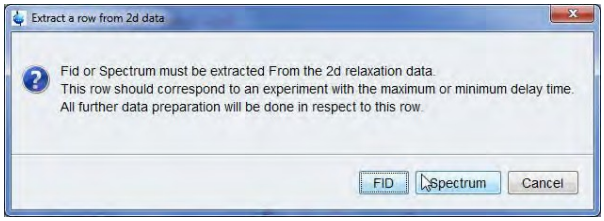


**PROCESSING**

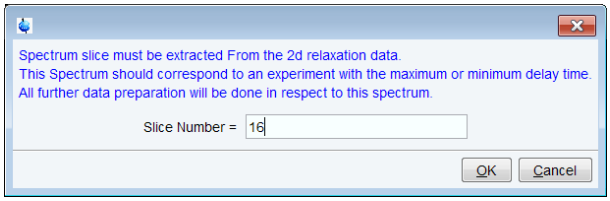
1. Process and adjust phase for the dataset. Use **rser n** (n is the number of total delays) to read out the last fid. Process and phase correct it. On the Adjust Phase toolbar, click **Save for spectrum**.


2. Go back to pseudo 2D T1 dataset by closing the 1D window
3. At the command prompt, type **xf2** to process only the F2 axis. Type **abs2** to baseline correct the rows.
4. On the menu bar, click **Applications**.
5. On the **Dynamics** button, click the drop-down arrow to see more options and in the list, select **T1/T2 Module**.

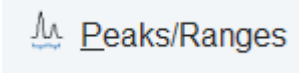

6. The flow buttons change to determine the T1 / T2 relaxation times. While executing the steps below, message windows will be displayed. Please read each message thoroughly and follow the instructions. On the Workflow button bar, click **Fid**
7. In the Extract a row from 2d data window, click **Spectrum**



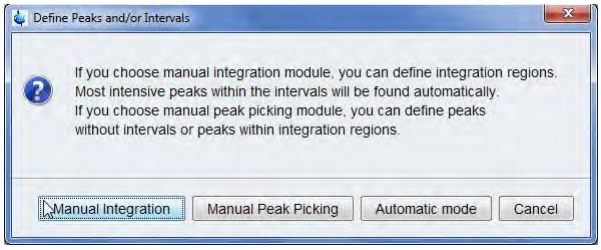

8. Enter Slice Number = **n** (the last one).



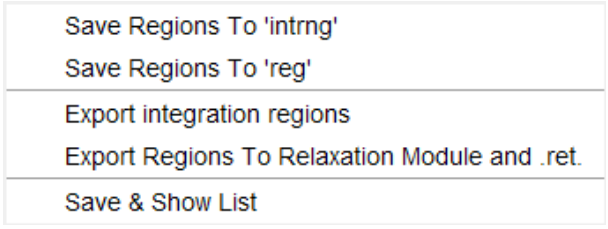

9. On the Workflow button bar, click **Peaks/Ranges**.



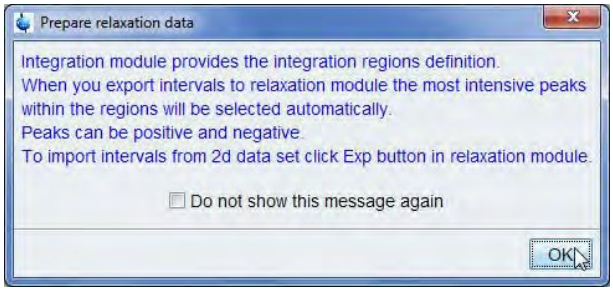

10. In the Define Peaks and/or Integrals window, click **Manual Integration**.

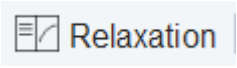


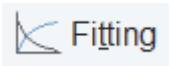

11. Define the regions by drawing an integral over the peaks of interest, On the Integration toolbar, click **Save/export integration regions** 
12. In the list, select **Export Region To Relaxation Module**.



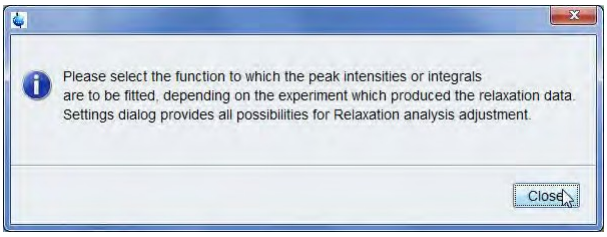

13. In the Prepare relaxation data window, click **OK**

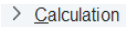



14. On the Workflow button bar, select **Relaxation**. 

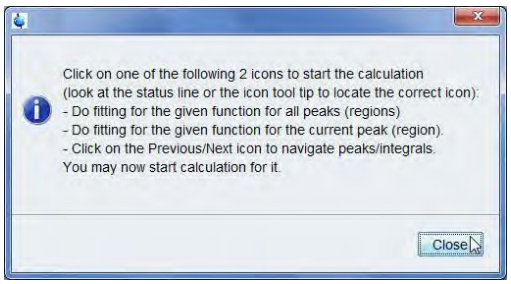

15. By default, the selected areas are peak-picked, and the first peak is displayed in the Relaxation window.
16. .On the Workflow button bar, select **Fitting** 

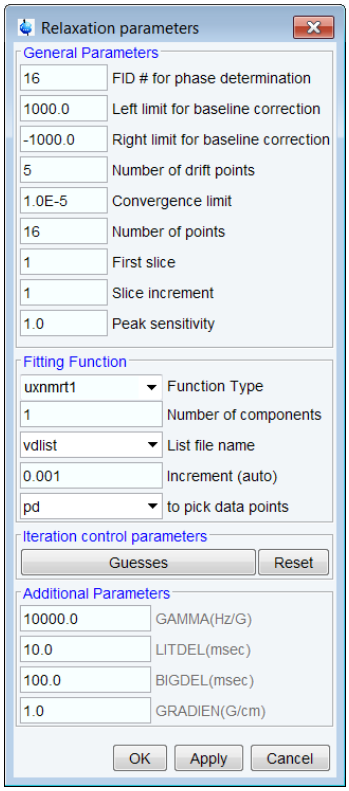
17. In the message window, click **Close**.



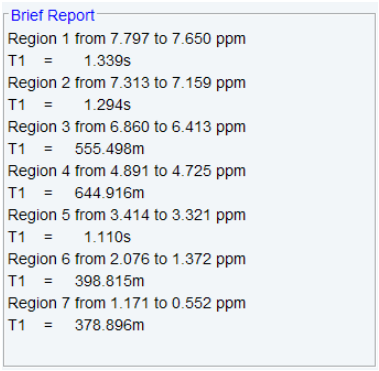
18. In the Relaxation parameters window, click **OK** and select **Area** as Fitting type.
19. On the Workflow button bar, select **Calculation**. 

20. In the message window, click **Close**.

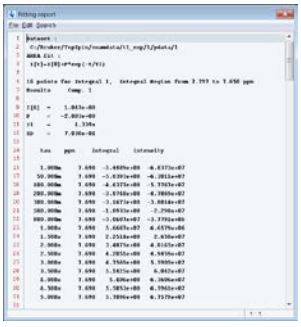



21. In the T1/T2 tools bar, click **Calculate fit for all peaks** 




22. On the Workflow button bar, select **Report** 

# APPENDIX F: KINETICS / REACTION MONITORING

## INTRODUCTION

As an intrinsically quantitative analytical technique, NMR spectroscopy can be used to measure concentrations of different components during chemical reactions for period of a few minutes to multiple days. One can either take a series of 1D spectra or acquire the data in pseudo 2D mode over the period of reaction. The best way to analyze the resulting data is to use MNOVA.

Before starting, you should have good estimates of the timeframe for your reaction and T1 relaxation time for your sample. Setup the **d1** and **aq** accordingly to make sure the relaxation time (**d1+aq**) is at least $5*T_1$. In general, the aliphatic protons on small molecules have T1 around 2 s and aromatic proton at about 4 s. You should run T1 experiment to get the numbers since it is very sample/solvent dependent.

1D method is a very versatile. Setup the 1D you will repeat and run **multi_zgvd**. It can be either a single pulse or single pulse with decoupling, i.e. F19 or F19CPD. The drawback of this method is that the timing between spectra can be off by a few seconds especially when your kinetics are fast and take less than an hour. The pseudo 2D, on the other hand, gives perfect timing for each fid, but it can be used only for single pulse experiment.

Once you decide how often you take a spectrum, you need strike a balance between the time resolution of the kinetic measurement and the amount of time needed to obtain sufficiently good signal-to-noise for each experiment. Limit the number of scans (ns) to be as small as necessary for adequate signal-to-noise to improve time resolution.

Before starting your reaction, please setup the experiment you want to repeat with a test sample with conditions similar to your real one. Do the locking, tuning, and shimming. Find the appropriate number of scans (ns) for adequate signal-to-noise. If resolving peaks is not a concern, you do not have to do lock/tune/shimming after putting the real sample in. Simply start acquiring the data, especially for F19.

## SEPARATE 1D SPECTRA W/ MULTI_ZGVD

This works for any nucleus, including proton.

1. Assume you already determined how often you want to run your recurring 1D experiment, let's call it **D20**, delay between start of different 1D spectra

2. Start with a normal 1D spectrum to adjust the spectral sweep width **SWH**, acquisition time **aq,** offset **O1p,** number of scans **NS**, and other parameter obtain sufficiently good signal-to-noise if necessary. Type **expt** to calculate how much time it takes. Let's call it "$\mathbf{T_{expt}}$". The delay between the end of one fid and start of next one equals **D20-$\mathbf{T_{expt}}$**. Let's call it $\mathbf{D_{fix}}$.
3. Create a new dataset with exactly same parameters from step 1. Start your reaction and load your sample to NMR instrument as fast as you can. Since you have already done locking/tuning/shimming on a test sample with similar conditions, you have following options:
   a) Do a topshim session first if your kinetics takes hours to finish.
   b) Skip the topshim if your reaction is really fast
4. Run **multi_zgvd**, when asked for a fixed or variable delay, answer with the default (fixed delay), then give the $\mathbf{D_{fix}}$ as the input for next question. For the question of "Enter number of experiments", give the numbers of experiment you want to run.
5. During the run, you can use **multiple display** to check peak intensity changes to evaluate if you reaction finishes or not.

**PSEUDO 2D MODE PROCEDURE**

The following procedure can be used for any nucleus.

1. Following step 1 and 2 of previous section to optimize the 1D experiment you want to repeat.
2. Create a new dataset and load the parameter set "**kx_zg2d_nu**".
3. Input the **D20** (delay between start of different 1D spectra) as shown in **Fig 1** and **TD** on F1 dimension (how many 1D spectra you want to acquire) as shown in **Fig 2.**
4. Start your reaction and load your sample to NMR instrument as fast as you can. Since you have already done locking/tuning/shimming on a test sample with similar conditions, you have following options:
   a) Do a topshim session first if your kinetics takes hours to finish.
   b) Skip the topshim if your reaction is really fast
5. Start your experiment by typing **zg** or click on "**run**".
6. During the run, you can use **rser** to check each individual fid as long as it is finished. For example, "**rser 1 10**" will write the 1st fid to experiment number 10; "**rser 20 11**" will write the 20th fid to experiment number 11. Then you can use **multiple display** to stack or superimpose them.

**Fig 1**. ACQUPARS display in “pulse program parameters” view

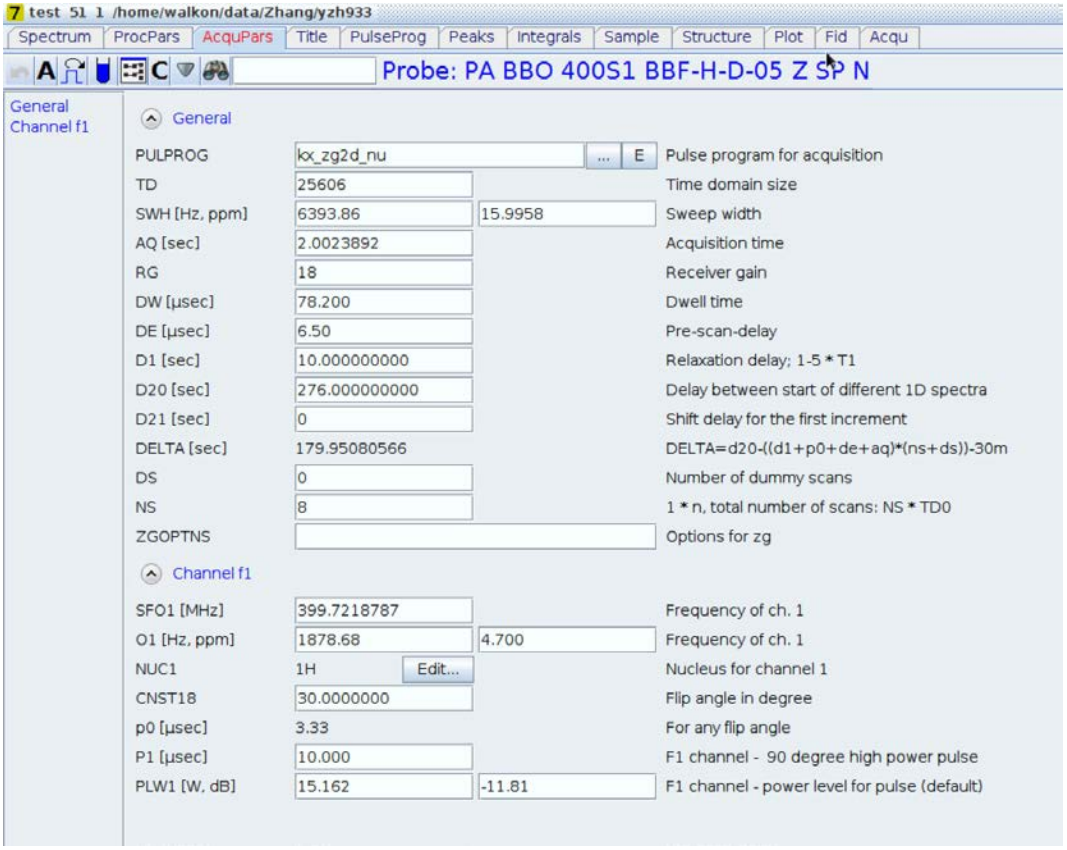
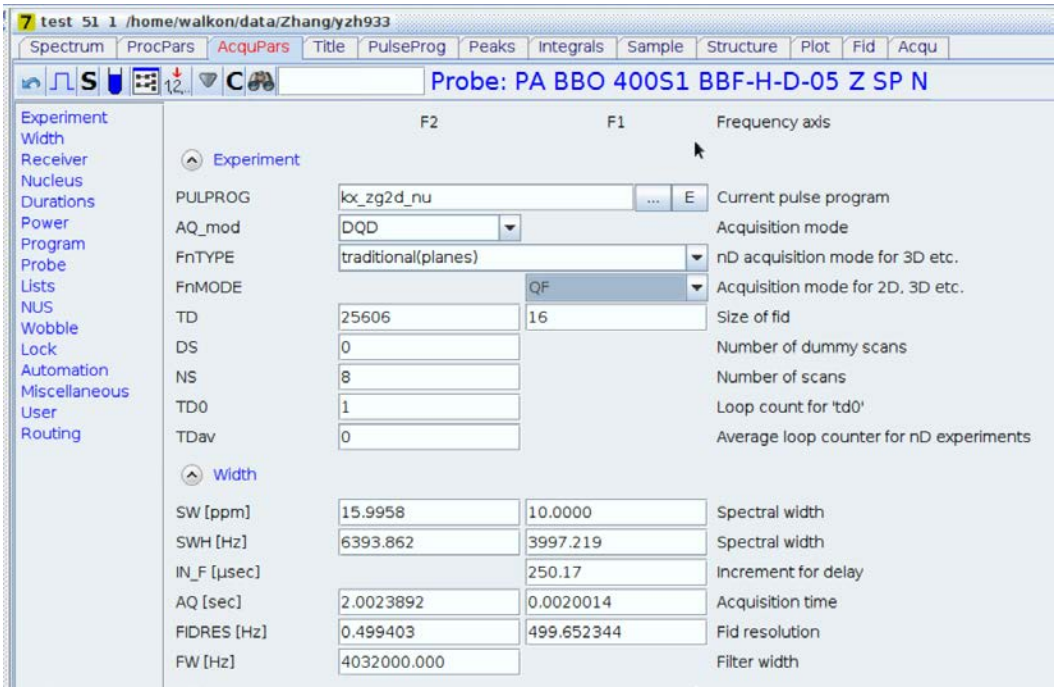



**Fig 2**. ACQUPARS display in “all acquisition parameters” view



## APPENDIX G: EVANS METHOD

### INTRODUCTION

Evans Method was established in 1959 for magnetic susceptibility measurement [Ref 1]. It was developed by many other researchers in the past half century.

This Evans Method manual will help NMR users understand paramagnetic susceptibility measurement by using NMR. Users can simply follow the procedures to conduct their experiments with little or even no NMR staff assistance

Now, the most common equation is (1) below

$$\chi_{mass} = 3\Delta f/4\pi fm + \chi_0 + \chi_0(d_0 - d_s)/m \qquad (1)\ [\text{Ref 2}]$$

- $\chi_{mass}$ is mass Susceptibility in $cm^3g^{-1}$
- $\Delta f$ is obs freq diff in Hz
- f is spectrometer freq, e.g. $399.732 \times 10^6$ Hz on Hg400
- m is mass of paramagnetic substance in $g \cdot cm^{-3}$ i.e. concentration in g/mL
- $\chi_0$ is mass susceptibility of solvent in $cm^3g^{-1}$
- $d_0$ is density of solvent in $g \cdot cm^{-3}$
- $d_s$ is density of solution in $g \cdot cm^{-3}$

There are some other related concepts: [Ref 3]

volume Susceptibility ($\chi_v$) $\chi_v = \rho\chi_{mass}$ here ρ is the density in $kg \cdot cm^{-3}$ or $g \cdot cm^{-3}$ and

molar Susceptibility ($\chi_{mol}$) $\chi_{mol} = M\chi_{mass}$ here M is molecular weight

Also, understand Conversion of SI and cgs units: $\chi^{SI} = 4\pi\chi^{cgs}$

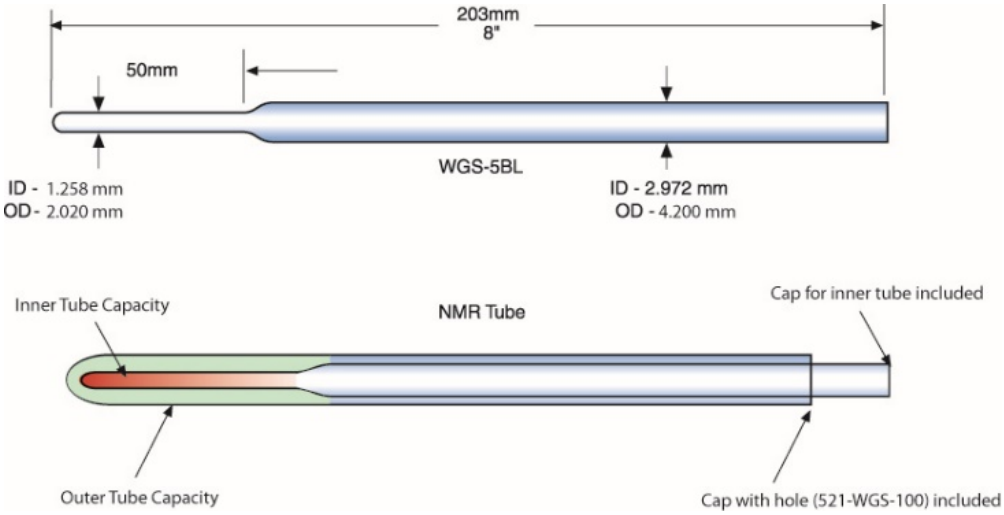
In this manual, we use cgs unit through-out. For instance, $\chi_{mol}$ in $cm^3mol^{-1}$

### PREPARATION

**Prerequisite**: users have done the basic NMR training

**Spectrometer**: NMR-Au400 recommended

**NMR tube**: coaxial inserts (see the picture below) or capillary inserts [Ref 4]




**NMR Sample Prep**: (using FeSO4, t-butanol and D2O as example [Ref 5])

- Solution A: dissolve 7.0 mg FeSO4.7H2O in 0.5 mL D2O, add 30 uL t-butanol, and adjust total volume to 1 mL with D2O
- Solution B: 30 uL t-butanol + 970 uL D2O
- Transfer A into the coaxial insert and B into a 5mm tube

**EXPERIMENT SETUP (QUICK PROCEDURES)**

1. Turn on spectrometer computer monitor by login NUcore with netid/password
2. Create a new data set and Set up a regular 1D 1H experiment
3. Insert NMR sample into magnet with SX
4. Do locking, tuning and shimming as usual
5. Run a regular 1D H1 (will see two methyl peaks caused by paramagnetic reagent)

**DATA ANALYSIS**

1. measure distance between two methyl peaks (Δf 510 Hz)
2. use a simplified equation $\chi_{mass} = 3\Delta f/4\pi fm + \chi_0$ (2)

   Here, f = 399.732 on Hg400

   m = 0.0072 g (actual weight) in 1.00 mL (solution A)

   $\chi_0$ is approximately equal to water mass susceptibility, $-0.72 \times 10^{-6}$ $cm^3/g$
3. molar susceptibility $\chi_{mol} = M\chi_{mass}$

   Here, molar mass M is 278 for FeSO4, and $\chi_{mol} \sim 11200 \times 10^{-6}$ [Ref. 6]
4. Actual experimental result is $\chi_{mass} = 41.58 \times 10^{-6}$ $cm^3/g$ and $\chi_{mol} = 11600 \times 10^{-6}$ $cm^3/mol$
5. If the $\chi_{mol}$ value is small, you may consider the diamagnetic susceptibility correction term

   $\chi_{mol\text{-}para} = \chi_{mol} + \chi_{mol\text{-}dia}$ [ref 7]

**REFERENCES**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## REVISIONS

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|---|---|
| V1.0<br>2020/2/2 | • Initial release. |
| V1.1<br>2020/2/10 | • Added 'Troubleshooting' section |
| V2.0<br>2020/4/28 | • Added additional specialty NMR experiments |