Setting up NMR Experiment in HFNMRC

2022.04.27

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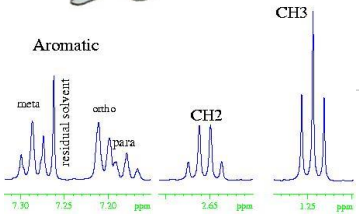
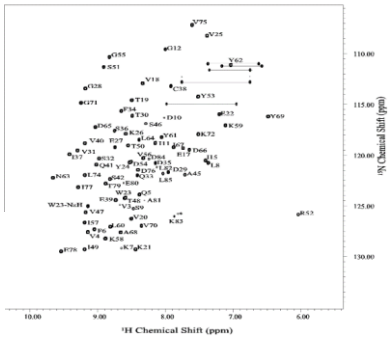
Steps for NMR Experiments

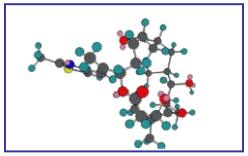
Prepare Samples → **Collect NMR spectra** → **Data Analysis**

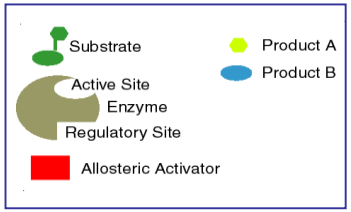
Collect NMR spectra
- Expt. Methods
- Expt, Design

Data Analysis
- Processing / Analysis
- Assignment, Structure Calculation, Interpretation
- ……..









Question : Which NMR ?

Which NMR is the best for my sample?

High Field 高磁場核磁共振中心
Nuclear Magnetic Resonance Center

HFNMRC HOME

NMR Spectrometers
Time reservation table

AVANCE 500 AV
(IBMS)

AVANCE III 850
(IBMS)

AVANCE III 600
(IBMS)

NEO 600
(IBMS)

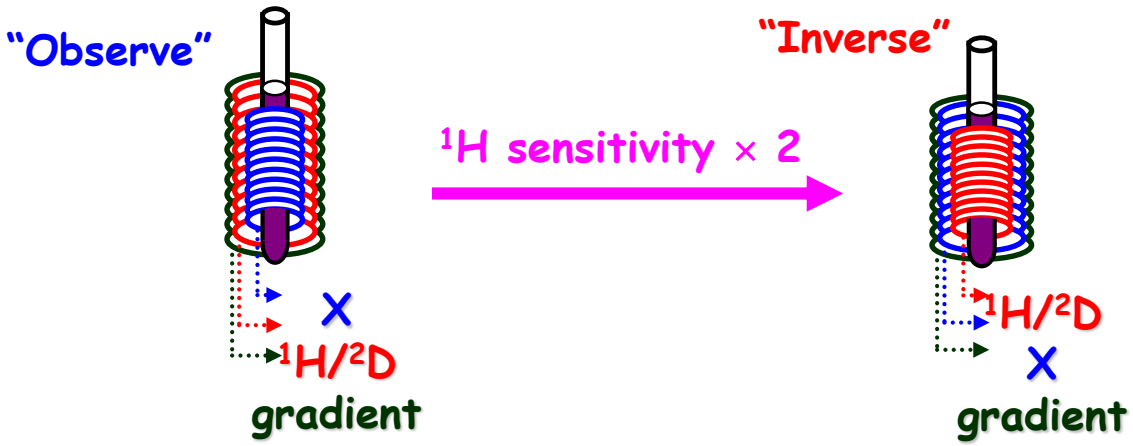
AVANCE 800 AV
(IBMS)

AVANCE 600 AV
(CHEM)

QA1: Which Probe could provide information I need?

Probe Type	Regular	Cryoprobe
Sensitivity* (organic solvent)	1	~4
(aqueous solution)	1	~2.5

Probe Coil	Observe (ex:TBO,BBO,Dual)	Inverse (ex: TXI, TCI)
	X-nuclei observed Experiment (1D 13C, 1D 31P)	1H-detected Experiments (1D 1H, 2D COSY/TOCSY 2D HSQC/HMBC)

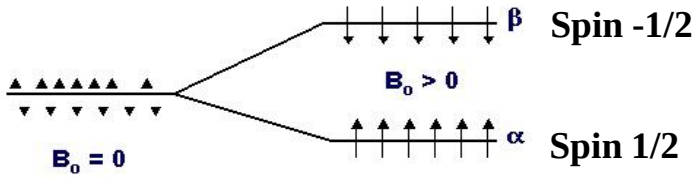


NMRs in HFNMRC

NMRs	Location	Probes	Note *current installed probe
AVIII850	IBMS B2	TXI_regular TCI_Cryo*	1H/13C/15N
AV800	IBMS B2	TXI_regular TXI_Cryo*	1H/13C/15N; with SampleJet -3 position for regular -96 positions for spinner-free -5 positons for rack (96 well-plate)
AVIII600	IBMS B2	TCI_Cryo*	1H/13C/15N
NEO600	IBMS B2	TCI_Cryo*	1H/13C/15N; with SampleCase (24 samples)
AV600_CHEM	CHEM B1	TXI/QXI_regular TBO_regular* BBO_regular	1H/19F/BB (ex: 13C,15N, 31P)
AV500	IBMS B2	TXI_Cryo QNP_Cryo*	1H/13C/31P/19F; with SampleXpress (60samples)

QA2: Do I need high field for better resolution ?

NMR field	<= 500MHz	>= 600 MHz
chemical shift(ppm)	the same	
coupling constant(J, Hz)	the same	
Sensitivity*	lower	higher
Resolution**	lower	better



$N_\beta/N_\alpha = \exp(-\Delta E/kT) = \exp[(\gamma h B_o)/(2\pi kT)]$

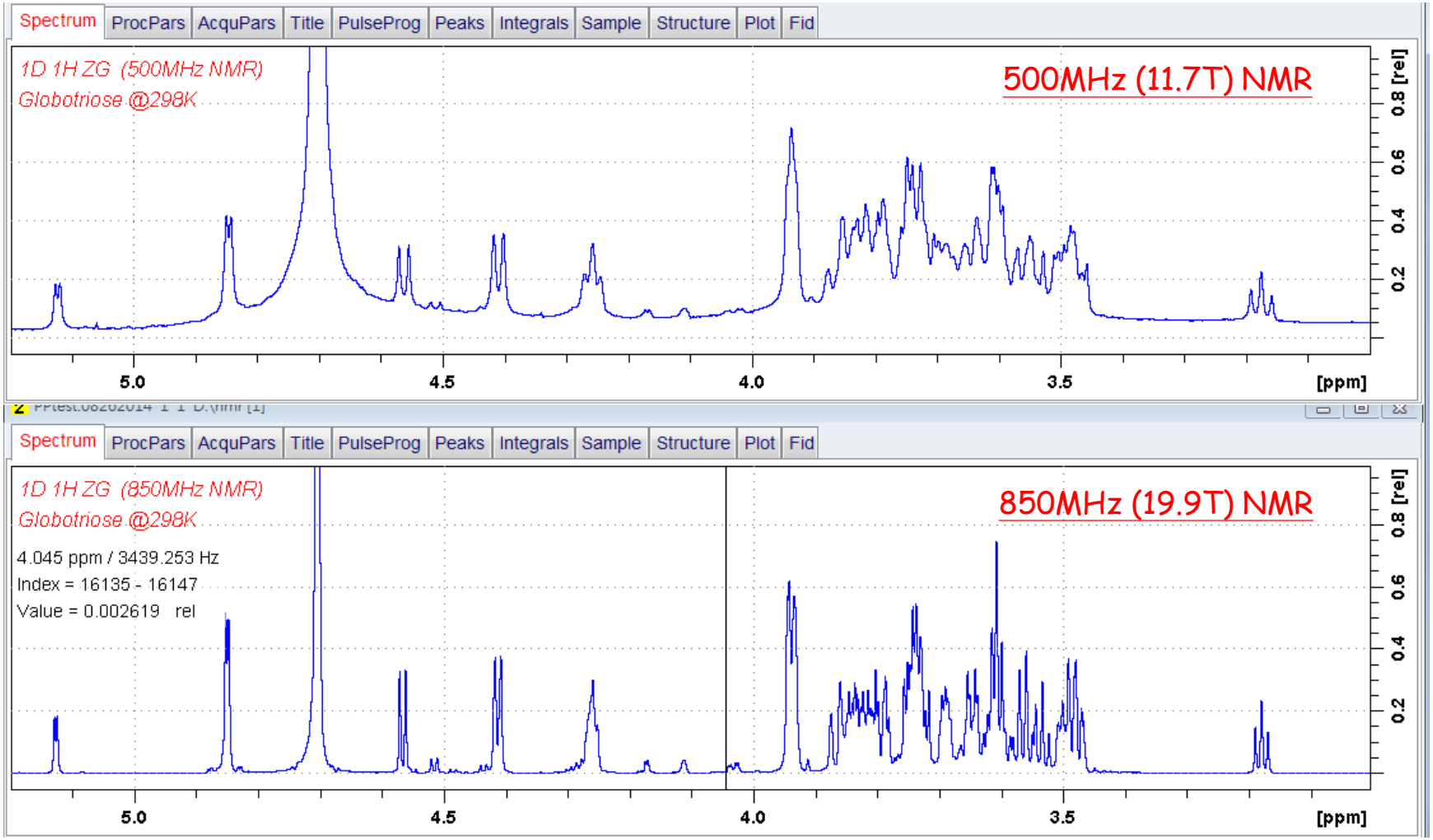
$$S/N = \frac{N\gamma_{exc}T_2(\gamma_{det}B_0)^{3/2}\sqrt{ns}}{T}$$

S/N	=	signal to noise ratio
N	=	number of spins in the system (sample concentration)
γ_{exc}	=	gyromagnetic ratio of the excited nucleus
γ_{det}	=	gyromagnetic ratio of the detected nucleus
ns	=	number of scans
B_0	=	external magnetic field
T_2	=	transverse relaxation time (determines the line width)
T	=	sample temperature

* Higher Sensitivity is needed for low concentration sample

** Better resolution is needed for overlap peaks

1D 1H Spectrum in ppm



Higher the field, better the sensitivity and resolution

NMR Probes S/N ratio in HFNMRC

Regular Probe*	1H (EB)	Others
500MHz_TXI	450	
600MHz_TXI	1,218	
600MHz_BBO	465	465 (13C)
600MHz_QXI	1,193	85(31P)
600MHz_TBO		
800MHz_TXI	2,077	

CRYO Probe*	1H (EB)	Others
500MHz_TXI	4,196	
500MHz_QNP	2,000	1,000 (13C) 988 (31P)
600MHz_TCI_005	5,700	710(13C)
600MHz_TCI_121	6,530	950(13C)
800MHz_TXI	6,200	
850MHz_TCI	8,500	1,600(13C)

*Signal to Noise (S/N)value @ installed date

NMR & Probe	Topspin	1H (EB)	Others
AV500_IBMS (Cryo QNP)	TP2.x	2,000	1,000 (13C) 988 (31P) 1,000 (19F)
AVIII600_IBMS (Cryo TCI_005)	TP2.x TP3.x	5,700	710(13C)
NEO600_IBMS (Cryo TCI_121)	TP4.x	6,530	950(13C)
AV600_CHEM (regular TBO)	TP2.x	406	377(13C) 241(31P) 342(19F)
AV800_IBMS (Cryo TXI)	TP2.x	6,200	N/A
AVIII850_IBMS (Cryo TCI)	TP2.x TP3.x	8,500	1,600(13C)

Which NMR?

The best NMR for your sample!!

Question : How to collect NMR data?

Simple Operation Guide for HFNMRC Users

by Dr. Chi-Fon Chang for small molecules (2022.03.10 updated)

Simple Operation Guide for HFNMRC Users

by Dr. Chi-Fon Chang for small molecules (2022.03.10 updated)

PART I: Preparation (Software & Hardware)

<Lock> The 2H signal is collected by "lock channel" that operates in parallel with the principle channels. "**Lock**" maintain the center of 2H resonance at a constant frequency.

- Deuterated solvents are used to generate the signal to be detected and monitored by the lock system. The frequency and strength of this signal will depend on the solvent used.
- The lock system uses a receiver to monitor this deuterium frequency and makes adjustments to the magnetic field strength accordingly.
- The deuterium frequency is measured several thousand times per second. Hence, as long as the system is locked, the user can be confident that the field is maintained at a constant strength during acquisition.

***Important: you must lock the correct solvent, otherwise, the spectrum chemical shift might be incorrect.**

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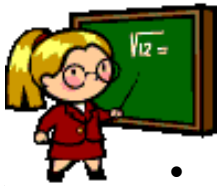
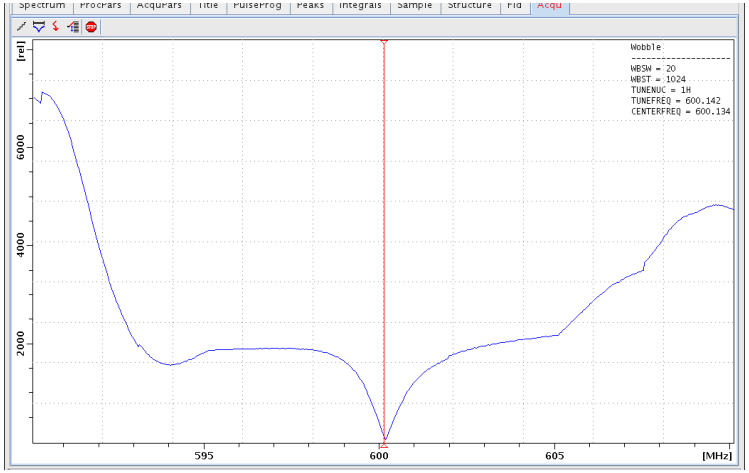
by Dr. Chi-Fon Chang for small molecules (2022.03.10 updated)

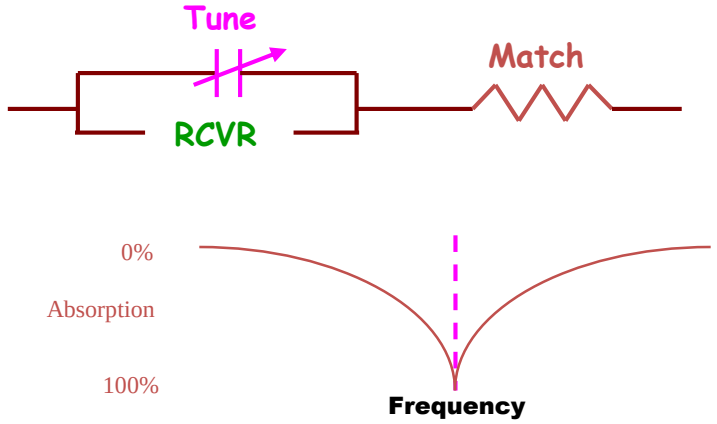
PART I: Preparation (Software & Hardware)



<Wobble> Wobble is to carry out "tuning" and "matching" simultaneously. Type "**wobb**" for old probe, "**atma**" or "**atmm**" for probe with ATM

- **Tuning** involves adjusting the probe circuitry so that the frequency at which it is most sensitive is the relevant transmission frequency (SFO1, SFO2 etc.)
- **Matching** involves ensuring that the maximum amount of the power arriving at the probe base is transmitted up to the coil which lies towards the top of the probe.





*Important: for QNP or BBO probe, you should wobble for the X-nuclei you like to observe

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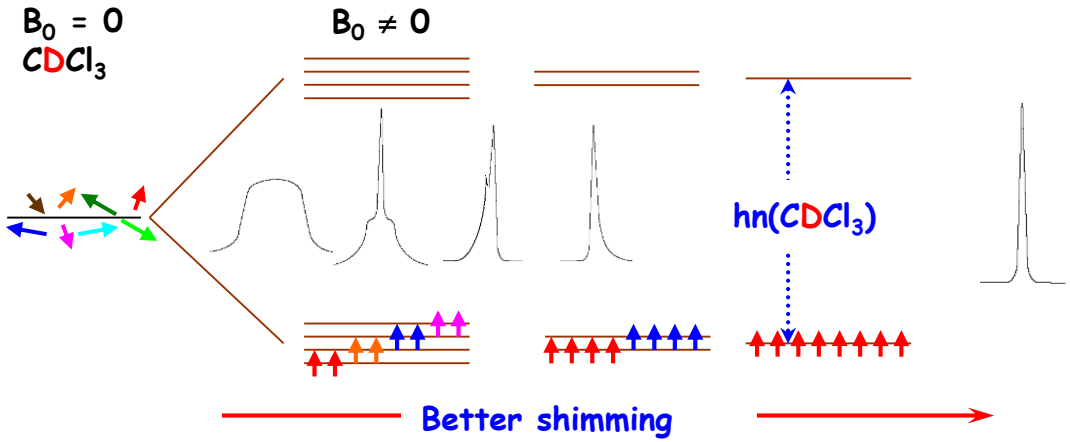
PART I: Preparation (Software & Hardware)

<Shimming> **Shimming** is a process in which minor adjustments are made to the magnetic field until the field homogeneity (uniformity) is optimized. Improving the homogeneity will result in better spectral resolution.

- Shimming is to adjust the shim coil circuits (ex: Z, Z2, Z3…X,Y….) which are designed to create small magnetic fields that will cancel out inhomogeneity in the Bo magnetic field.



- Type "**topshim**" for automation, manually shim might be needed

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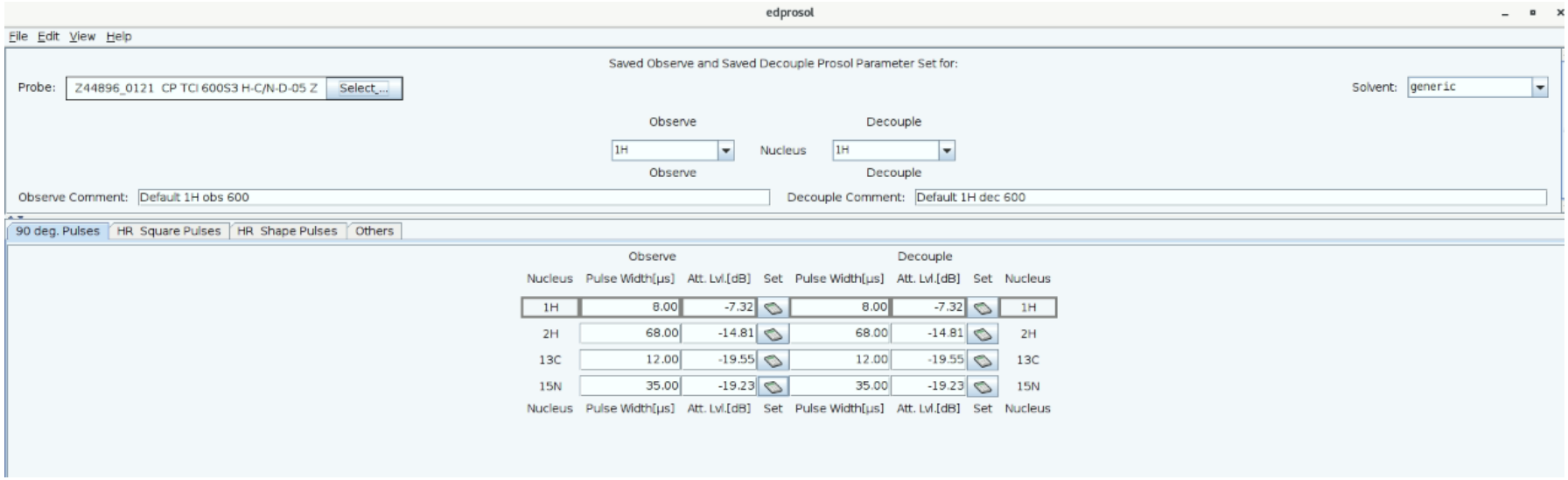
by Dr. Chi-Fon Chang for small molecules (2022.03.10 updated)

PART II: Experiment Set up & Data Collection

<getprosol> **getprosol** is to read in pulse parameters saved in the "prosol"

- **prosol** is a file (table) containing **Probe/Solvent** parameters (calibrated using standard sample, ex: Urea/Methanol) for the probehead installed on a specific NMR system.



*Important: the pulse parameters in "HFNMRC standard parameter set" won't be the same for current probe. Thus, you must "getprosol" to read in the correct parameters for current probe you are using.

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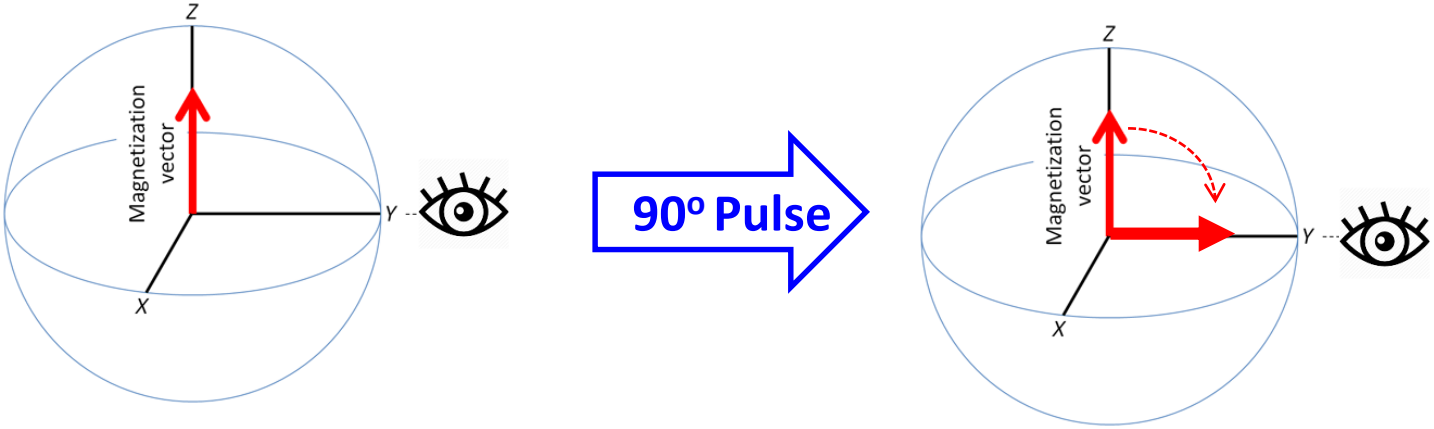
by Dr. Chi-Fon Chang for small molecules (2022.03.10 updated)

PART II: Experiment Set up & Data Collection

<pulsecal> is a command to determine 90 degree pulse for your sample

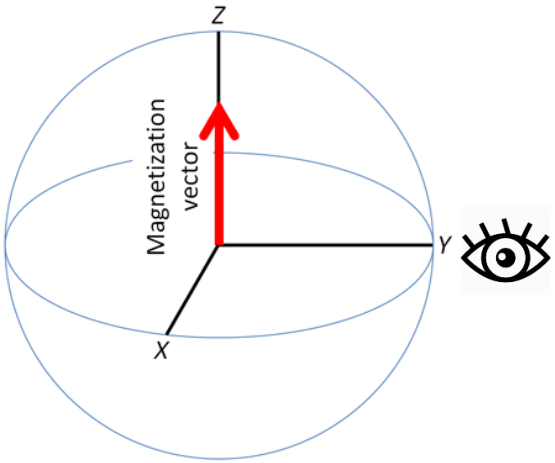


- Write down the power level (dB) and corresponding pulse-length(us)
- Instead of "getprosol" only, you should type

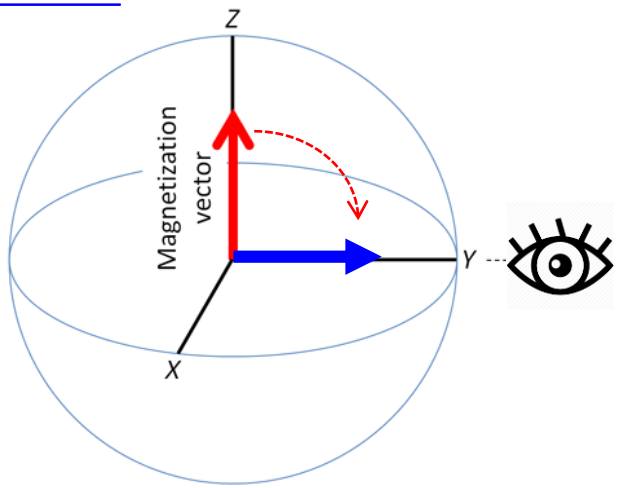
  "getprosol 1H <90° pulselength> < corresponding power level> "

*With optimized 90 degree pulse, the spectrum quality could be better. Especially for multi-dimensional experiments or experiments with specific pulse angles (ex: dept90, dept135, cosy45)!

90° vs. 30° Pulse

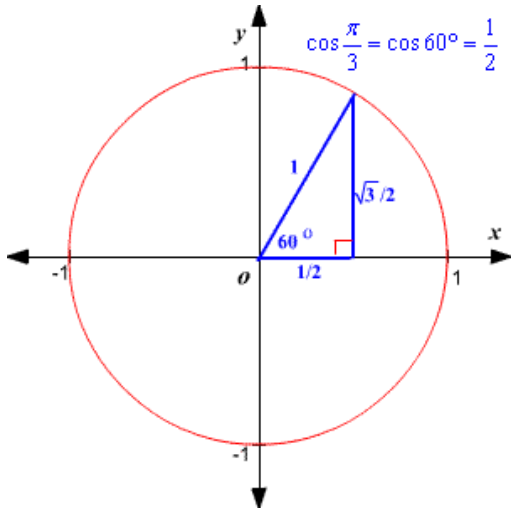


90° Pulse

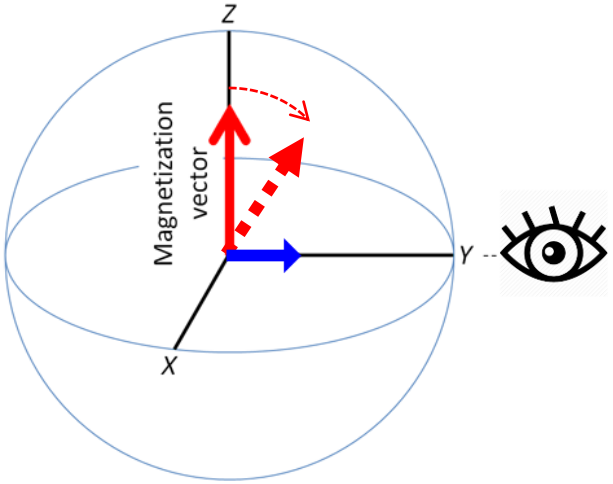


Signal Intensity=Full →

But need to wait longer time for next scan



30° Pulse



Signal Intensity=1/2 →

But back to Z faster, can collect more scan

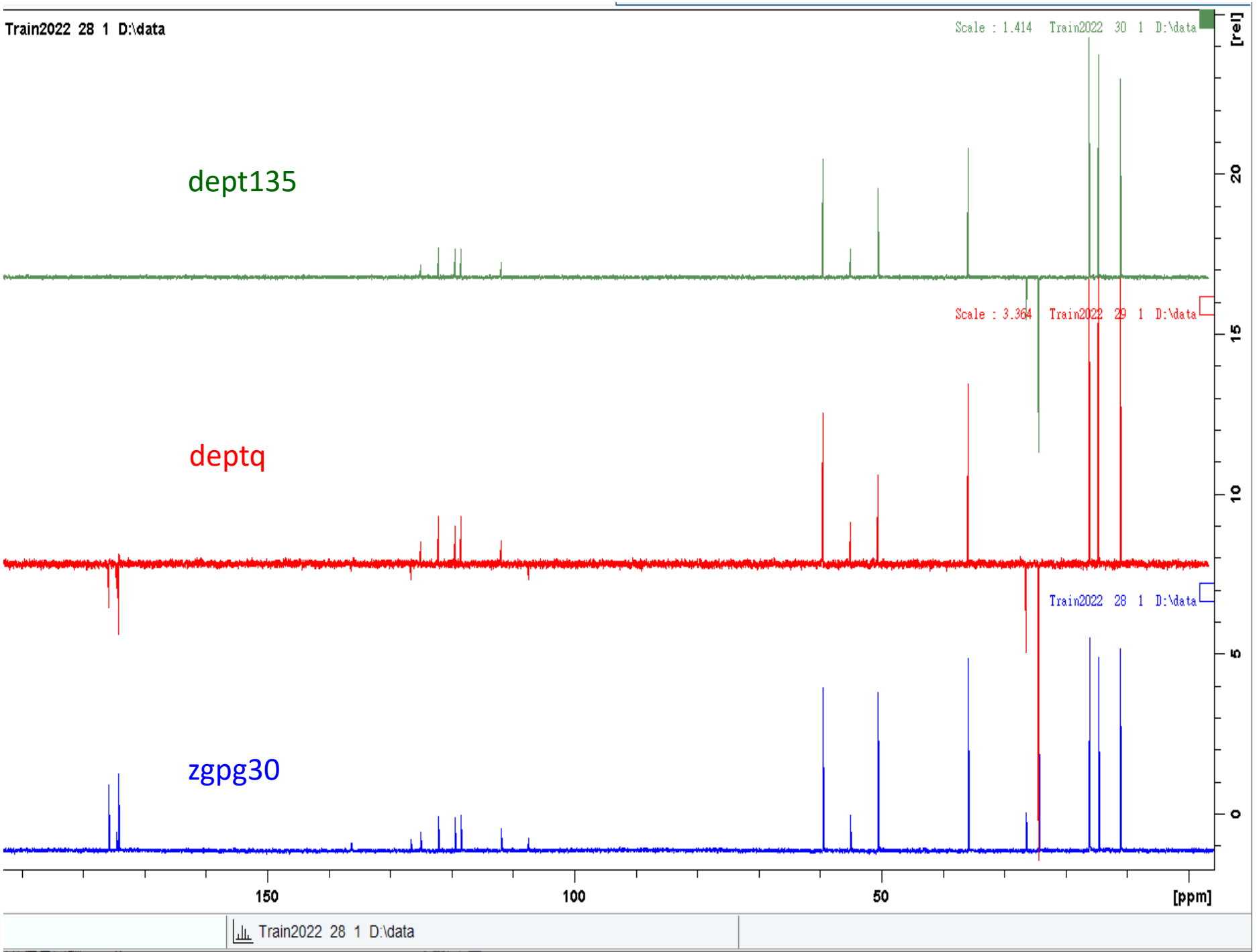
Hands-on
Let's try Some Experiments

- **1D 1H zggppr**
- **1D 13C deptq**
- **1D Selected TOCSY/NOESY**
- **2D 1H-13C HSQC using NUS**
- **Diffusion Experiments (DOSY)**

1 D 1H/13C Experiments

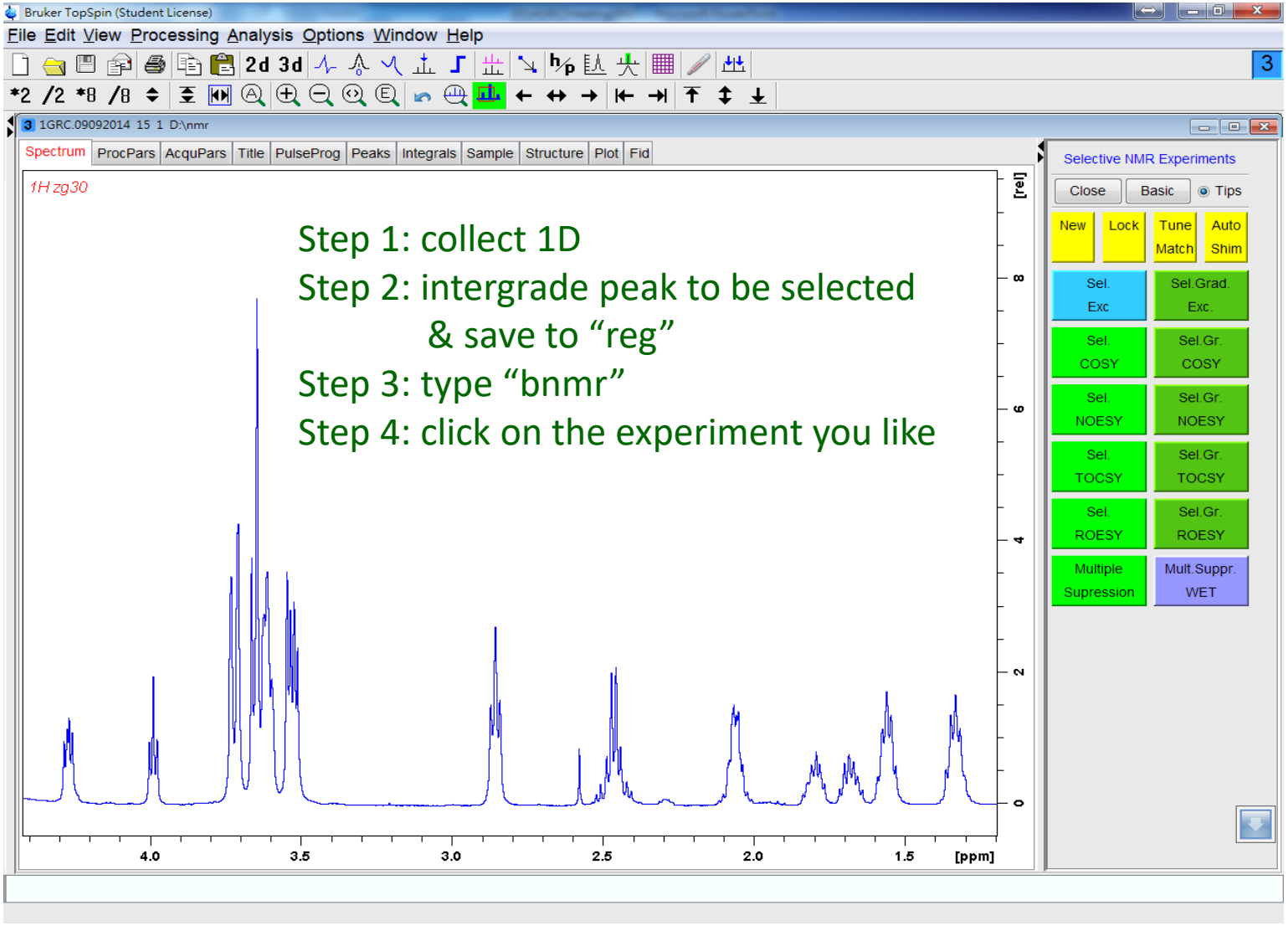
GRC Parameter Set	Experiment Details
1GRC_1D_1H-ZG-sol_zggppr	1H NMR with solvent suppression
1GRC_1D_13C-DEPTQ_deptqgpsp.2	Similar to DEPT135 but quaternary carbons are present

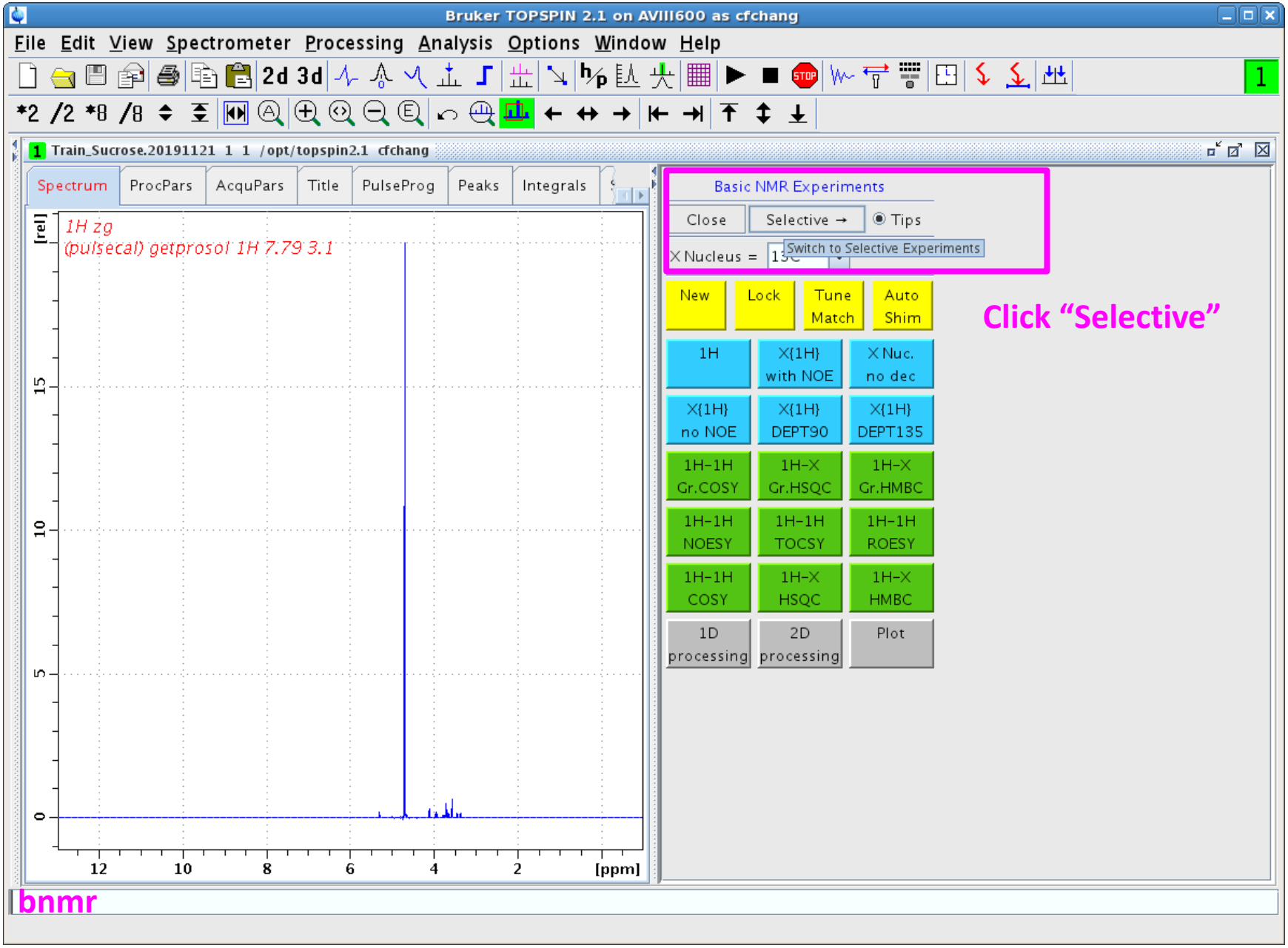
Expt	C	CH	CH2	CH3
DEPT45	NA	Positive	Positive	Positive
DEPT90	NA	Positive	NA	NA
DEPT135	NA	Positive	Negative	Positive
DEPTQ	Negative	Positive	Negative	Positive


Train2022 28 1 D:\data
Scale : 1.414 Train2022 30 1 D:\data
dept135
Scale : 3.364 Train2022 29 1 D:\data
deptq
Train2022 28 1 D:\data
zgpg30
[rel]
20
15
10
5
0
150
100
50
[ppm]
Train2022 28 1 D:\data

1D Selected Excitation Experiments

Use button NMR




Bruker TOPSPIN 2.1 on AVIII600 as cfchang
File Edit View Spectrometer Processing Analysis Options Window Help
Train_Sucrose.20191121 1 1 /opt/topspin2.1 cfchang
Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals
1H zg
(pulsecal) getprosol 1H 7.79 3.1
[rel]
[ppm]
Basic NMR Experiments
Close
Selective →
Tips
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Switch to Selective Experiments
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Lock
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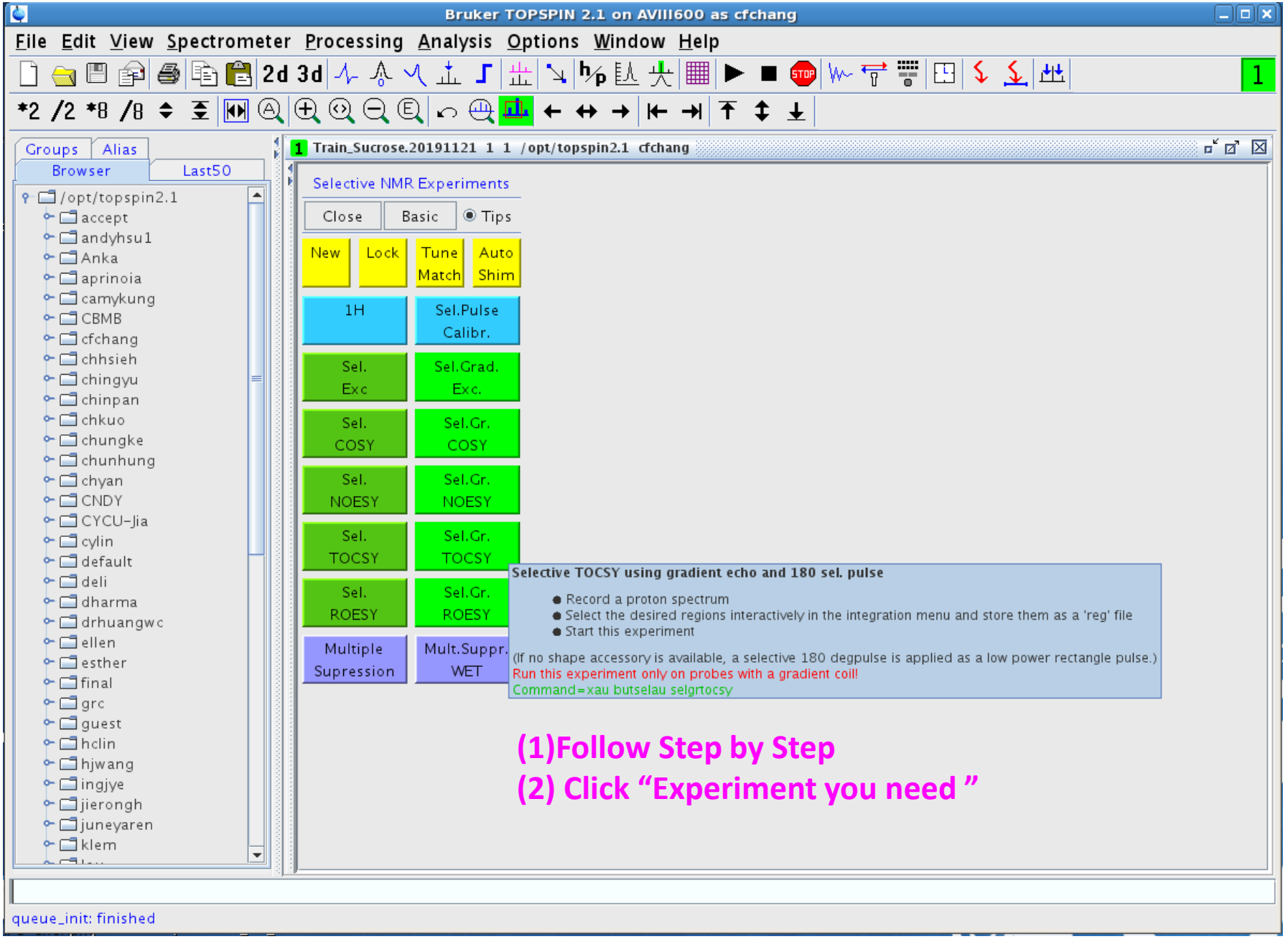
Auto Shim
1H
X{1H} with NOE
X Nuc. no dec
X{1H} no NOE
X{1H} DEPT90
X{1H} DEPT135
1H-1H Gr.COSY
1H-X Gr.HSQC
1H-X Gr.HMBC
1H-1H NOESY
1H-1H TOCSY
1H-1H ROESY
1H-1H COSY
1H-X HSQC
1H-X HMBC
1D processing
2D processing
Plot
Click “Selective”
bnmr


Bruker TOPSPIN 2.1 on AVIII600 as cfchang
File Edit View Spectrometer Processing Analysis Options Window Help
Groups Alias
Browser Last50
/opt/topspin2.1
accept
andyhsu1
Anka
aprinoia
camykung
CBMB
cfchang
chhsieh
chingyu
chinpan
chkuo
chungke
chunhung
chyan
CNDY
CYCU-Jia
cylin
default
deli
dharma
drhuangwc
ellen
esther
final
grc
guest
hclin
hjwang
ingjye
jierongh
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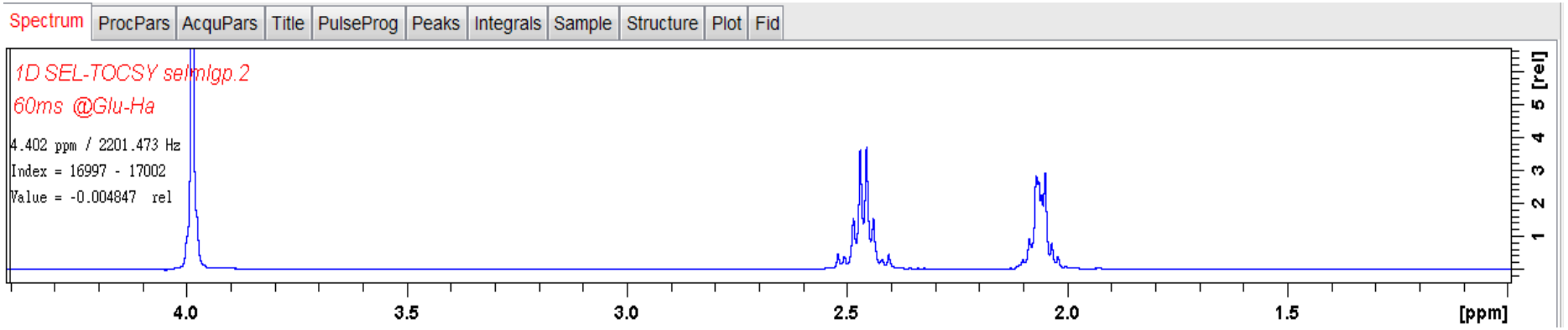
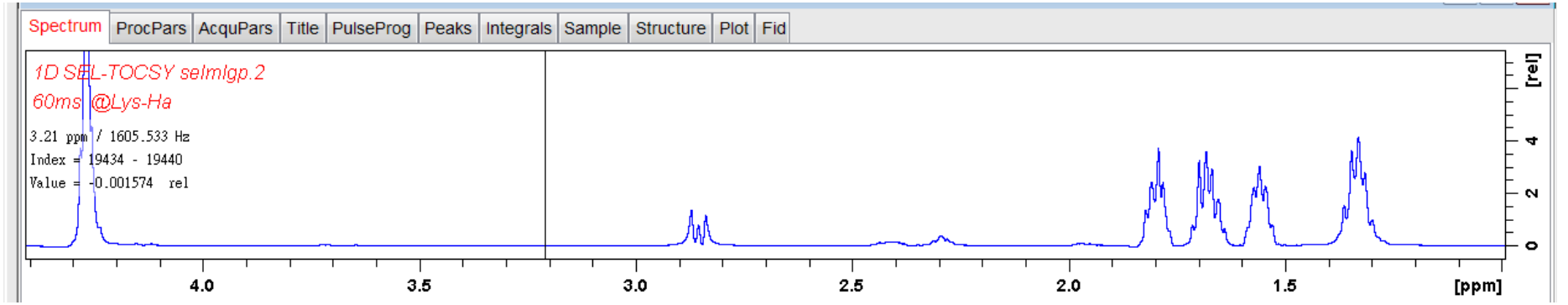
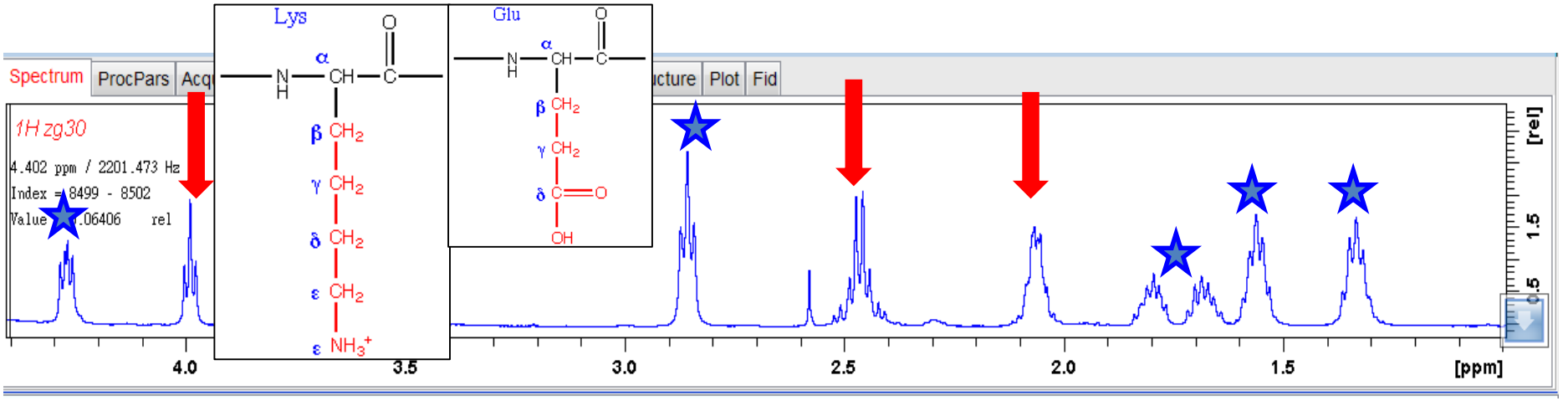
klem
1 Train_Sucrose.20191121 1 1 /opt/topspin2.1 cfchang
Selective NMR Experiments
Close
Basic
Tips
New
Lock
Tune Match
Auto Shim
1H
Sel.Pulse Calibr.
Sel. Exc
Sel.Grad. Exc.
Sel. COSY
Sel.Gr. COSY
Sel. NOESY
Sel.Gr. NOESY
Sel. TOCSY
Sel.Gr. TOCSY
Sel. ROESY
Sel.Gr. ROESY
Multiple Supression
Mult.Suppr. WET
Selective TOCSY using gradient echo and 180 sel. pulse
Record a proton spectrum
Select the desired regions interactively in the integration menu and store them as a 'reg' file
Start this experiment
(If no shape accessory is available, a selective 180 degpulse is applied as a low power rectangle pulse.)
Run this experiment only on probes with a gradient coil!
Command=xau butselau selgrtocsy
(1)Follow Step by Step
(2) Click “Experiment you need ”
queue_init: finished

1D Selected TOCSY

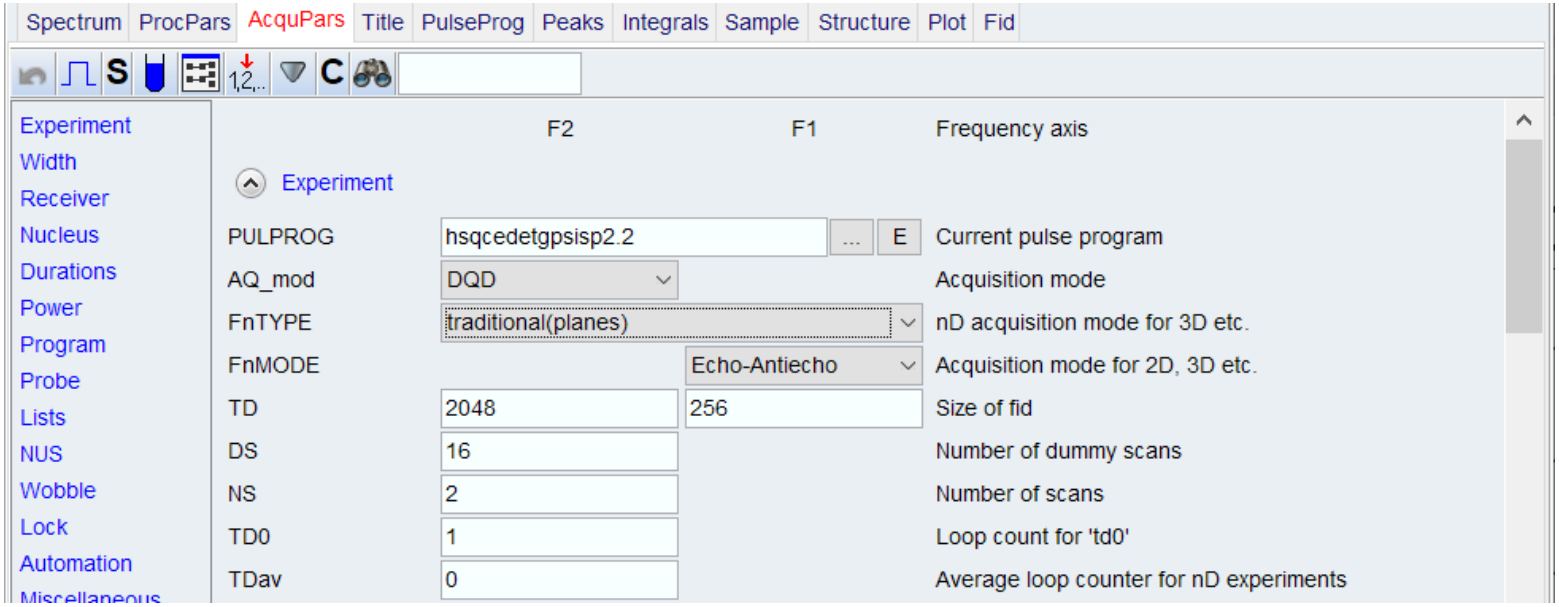




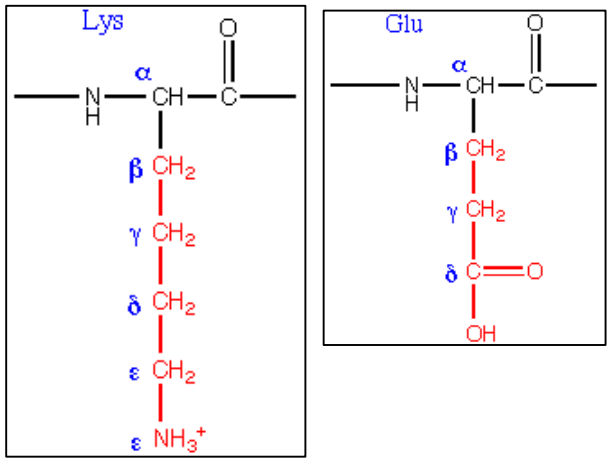


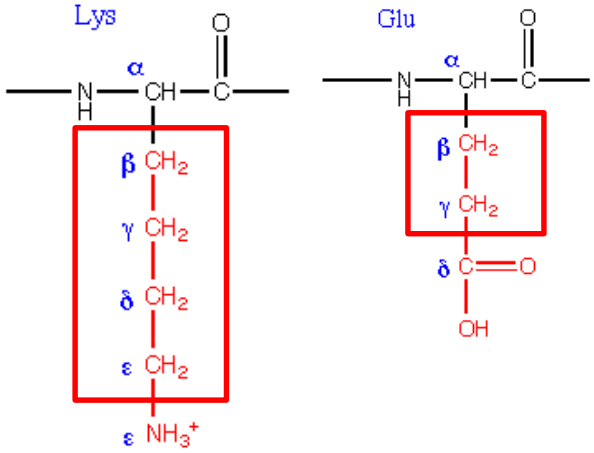
2 D 1H/13C HSQC

GRC Parameter Set	Experiment Details
1GRC_2D_HSQC_hsqcetgpsisp2.2	Routinely used
1GRC_2D_HSQC-editing_hsqcedetgpsisp2.2	Similar to DEPT135

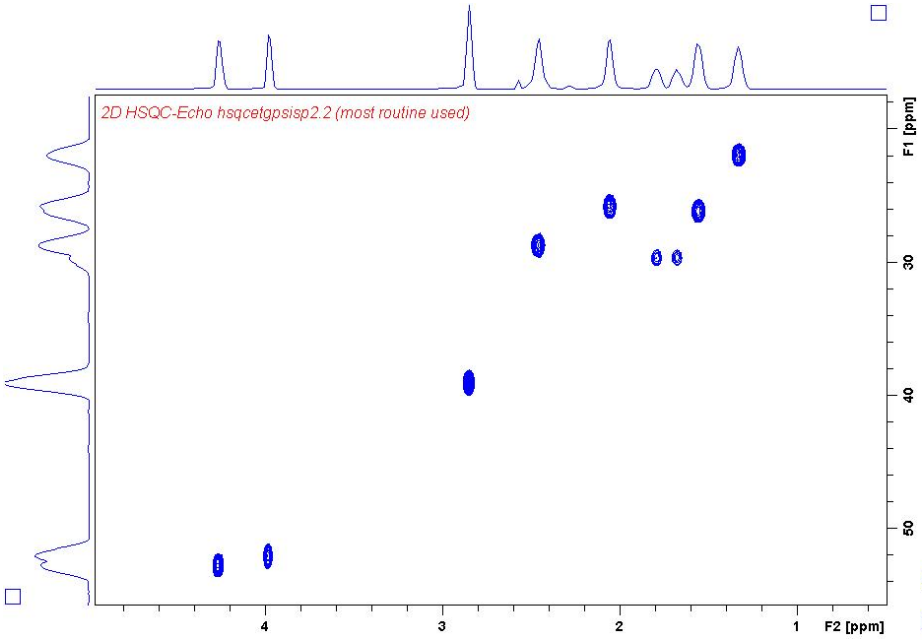


Example on different version HSQC

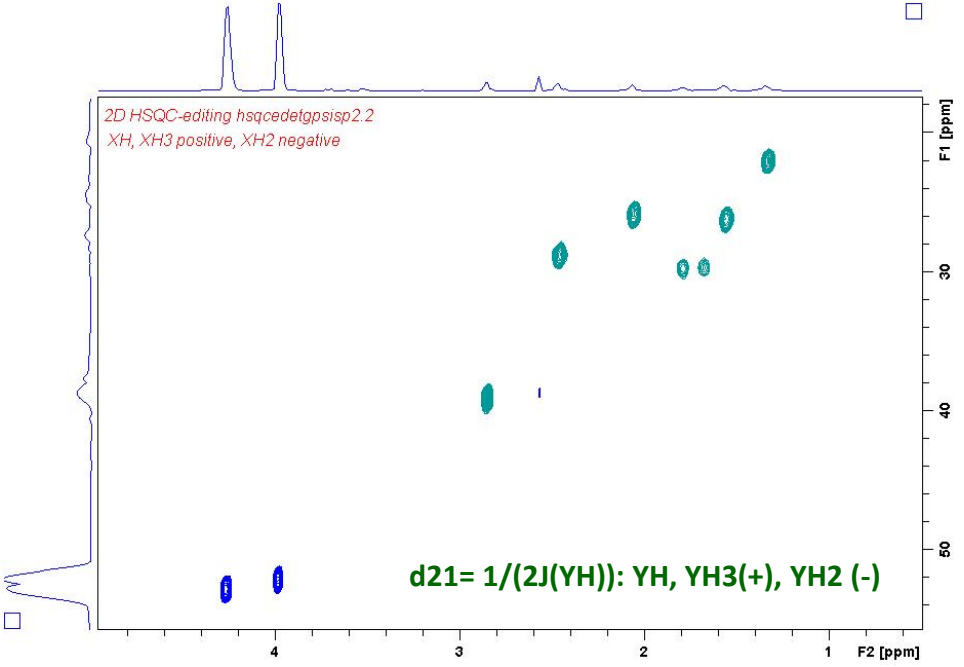




1GRC_2D_HSQC_hsqcetgpsisp2.2



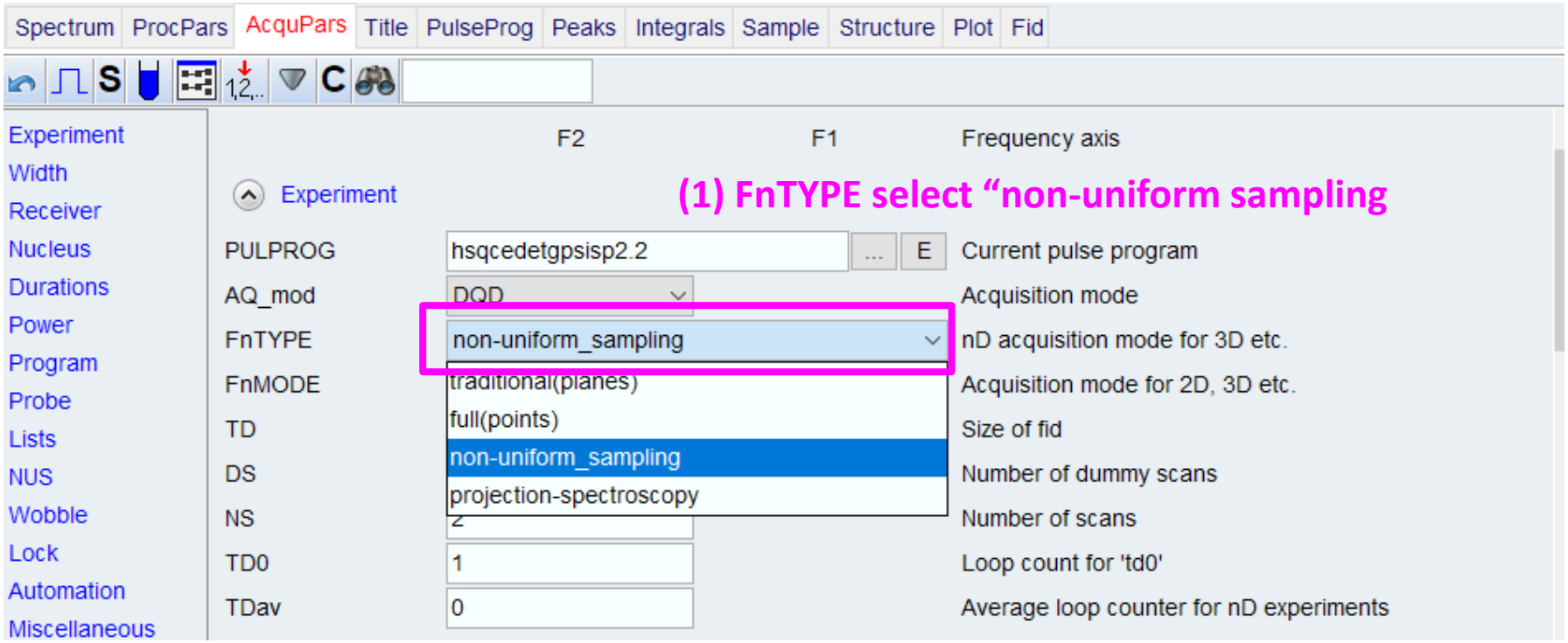
1GRC_2D_HSQC-editing_hsqcedetgpsisp2.2

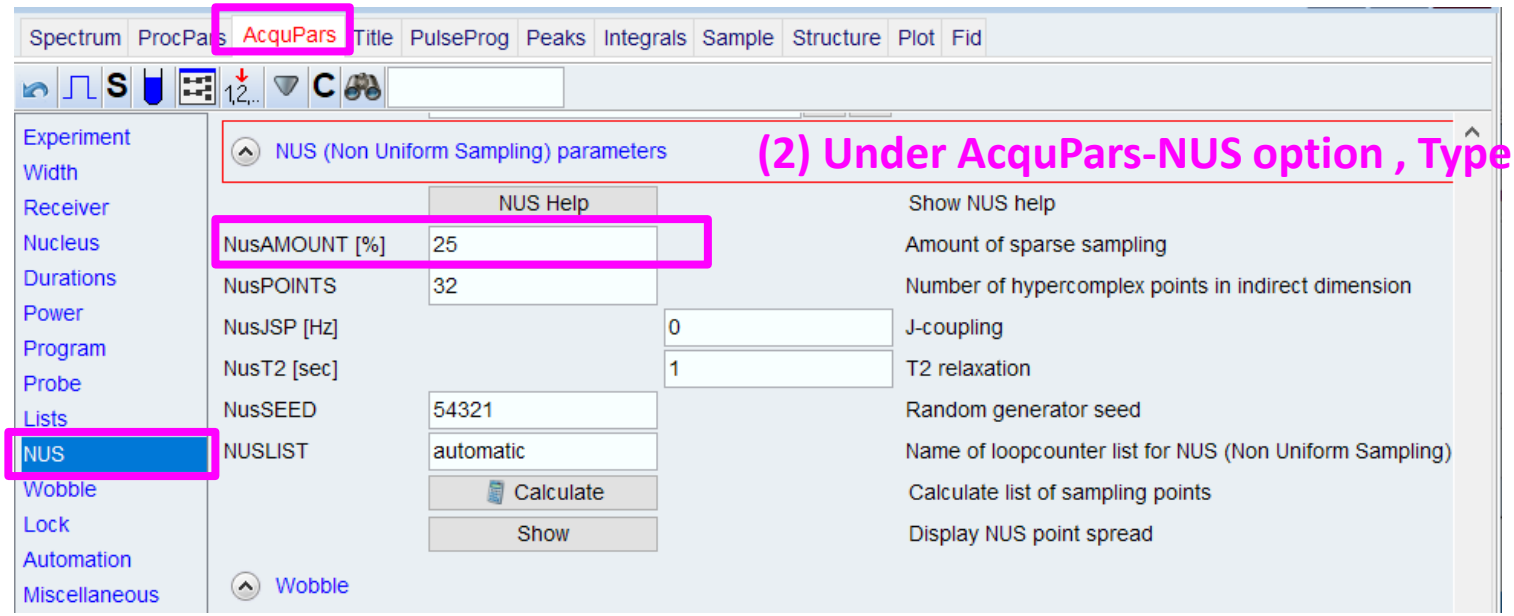


2 D 1H/13C editing HSQC with NUS

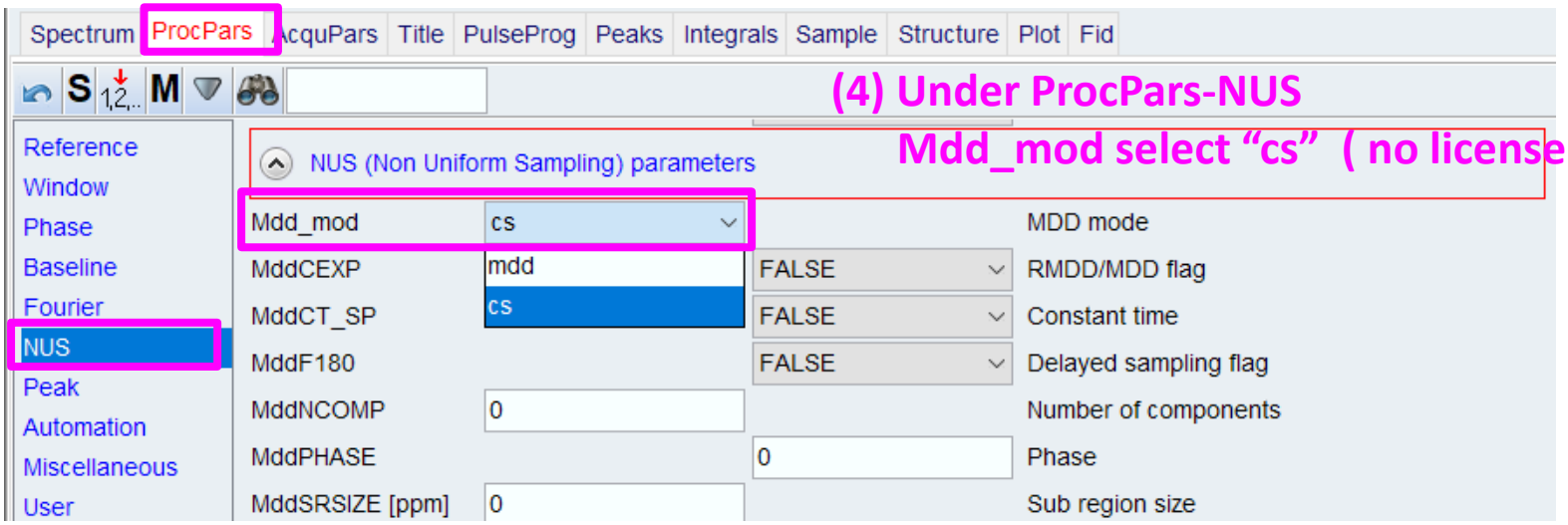
(Bruker NUS only available on Topspin3.x or above)

GRC Parameter Set	Experiment Details
1GRC_2D_HSQC-editing_hsqcedetgpsisp2.2	Similar to DEPT135





(3) zg to collect data



(5) xht2 to phase

(6) xfb for 2D processing

1D Diffusion Experiment

GRC Parameter Set	Experiment Details
1GRC_1D_DOSY-sol_ledbpgp2s1d	
1GRC_1D_DOSY-sol_ledbpgppr2s1d	With presat solvent suppression
1GRC_1D_DOSY-sol_stebpgp1s191d	With 3-9-19 solvent suppression

1D DOSY set up Tips:

(1) GPZ6=5 --> rga,zg,efp,apk, abs n --> wrp 2
(2) GPZ6=95 --> adjust d20 and/or p30 to scale ~5%-10% *
(3) Write down d20 and p30 for 2D

- If > 10%, increase d20 , after that, p30 can be increased, but keep p30<2ms (cryoprobes) <3ms (regular probe)
- If < 5% , decrease either d20 or p30

2D Diffusion Experiment

GRC Parameter Set	Experiment Details
1GRC_2D_DOSY-sol_ledbpgp2s	
1GRC_2D_DOSY-sol_ledbpgppr2s	With presat solvent suppression
1GRC_2D_DOSY-sol_stebpgp1s19	With 3-9-19 solvent suppression

2D DOSY set up Tips:

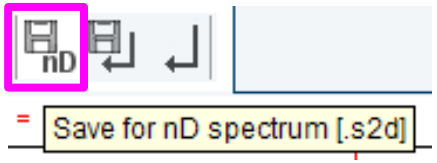
(1) Key in optimized d20 and p30 from 1D DOSY
(2) type" dosy" to set up experiment
    a. gradient amplitude from 5% -95%
    b. set number of points between 7-25 (default is 16)
    c. ramp type : q
(3) Start experiment

2D DOSY Processing Tips

(1) **SI[F1]= TD[F1]x2** ; TD[F1]= number of gradients, or larger (ex:128)

(2) Check phase

```
>rser 1
>efp
>abs n
>.ph
>save to nD          ; return to 2D data set
```



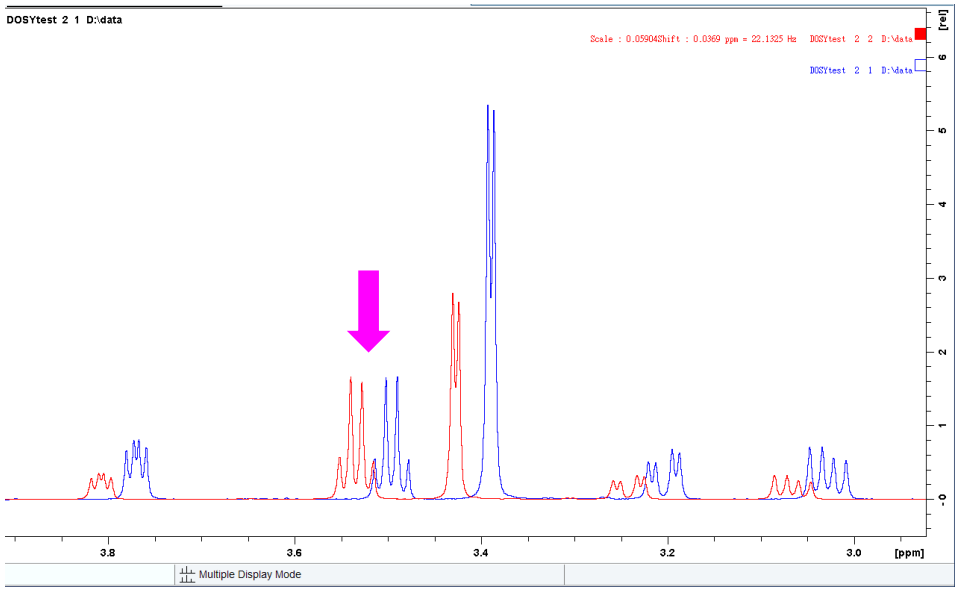
(3) Transform all FID in 2D

```
>xf2
>abs2
```

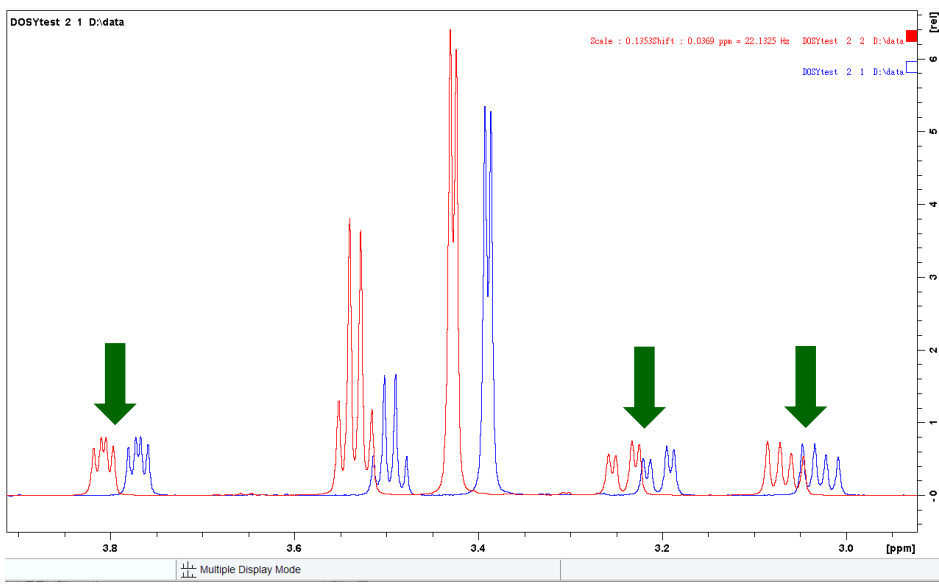
(4) Start DOSY processing

```
>setdiffparm        ;moves d20 and p30 into processing modules
>eddosy             ; you can change PC to larger number (ex: 10 or 40)
>dosy2d setup       ; run-through data and estimated D range
>dosy2d             ; performs DOSY transform as setup in eddosy
```

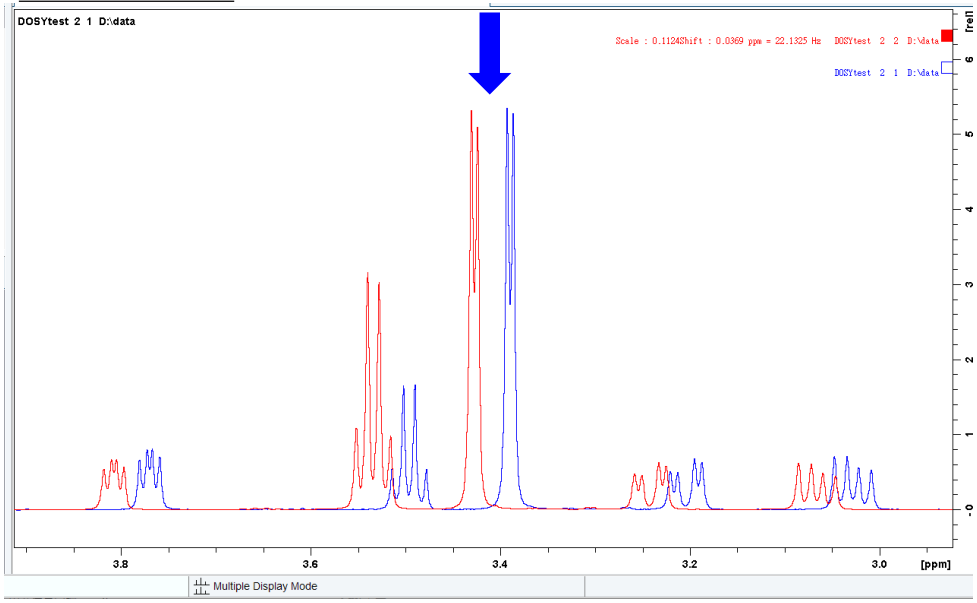
1D DOSY



1D DOSY



1D DOSY



2D DOSY