2023 NMR Users Training (II)

Basic NMR SOP for
Small Molecules & Metabolomics Analysis

Useful Experiments for Small Molecules

Chi-Fon Chang
Manager, HFNMRC
Senior Research Specialist/Scientist, GRC
Academia Sinica
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Useful Experiments for Small Molecules

- **Compound identification**
- **Structure Elucidation**

HFNMRC NMR Data Collection Service Request Form

10.測定項目（**NMR Measurement**）：

一維圖譜（1D） ☐^{1}H（☐decouple）；☐^{13}C（☐decouple） ☐其他（Others）_____

二維圖譜（2D） ☐COSY ☐TOCSY ☐ROESY ☐NOESY ☐HMQC ☐HSQC ☐HMBC ☐其他（Others）_____

其他圖譜 (Other Experiment) _____

_____

實驗方法 (Acquisition Mode) ☐ Regular ☐ Non-uniform Sampling

化學位移（Chemical Shift） ^{1}H_____ppm，^{13}C_____ppm，其他 (other)_____

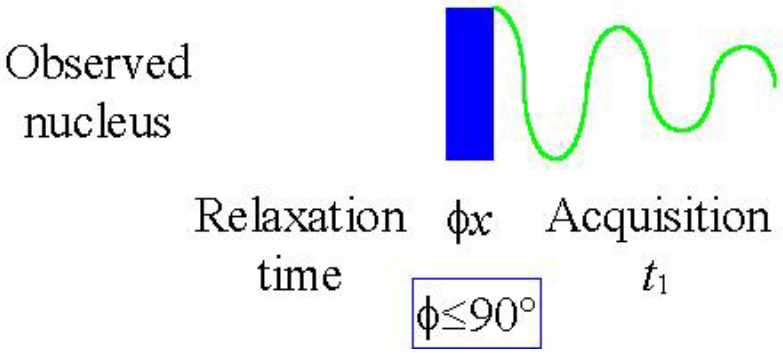
溫度條件（Temp. Condition）_____

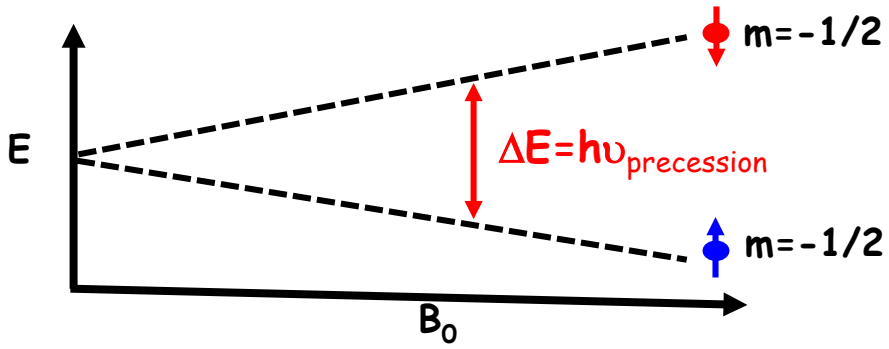
11. 預估分子量或結構 (molecular weight & structure information if available)：

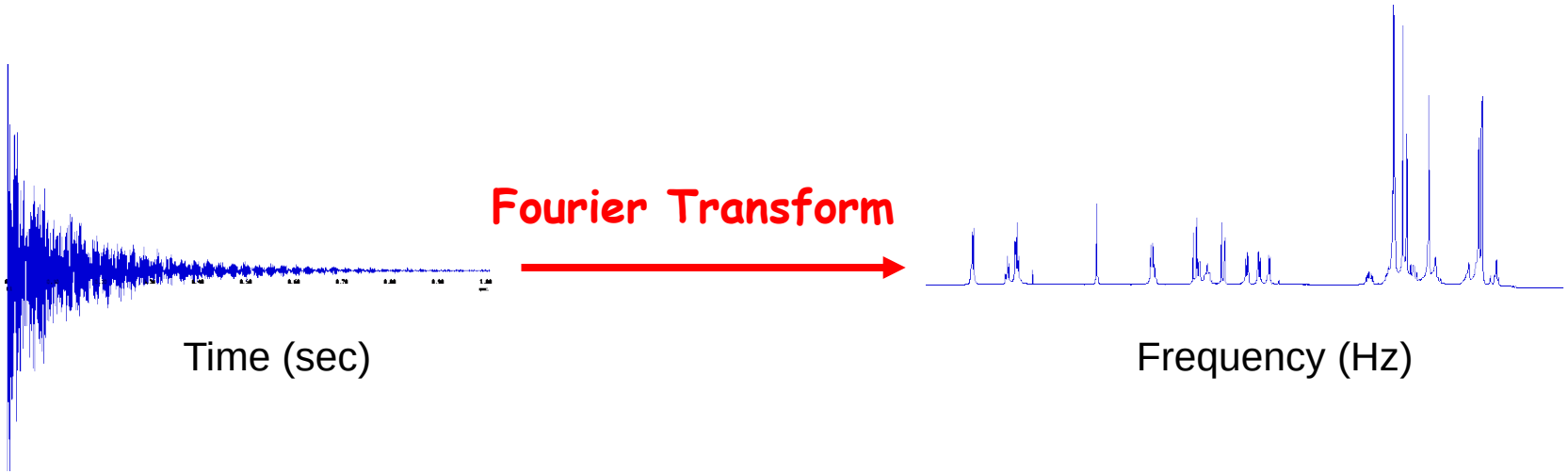
Let's see what information NMR Experiments can tell us ?

工欲善其事，宜先知其器

1D one pulse NMR Experiment

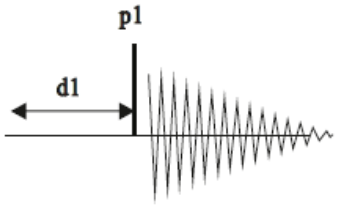




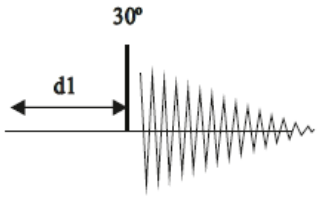


1D ZG

zg

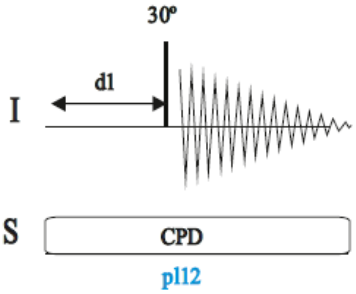


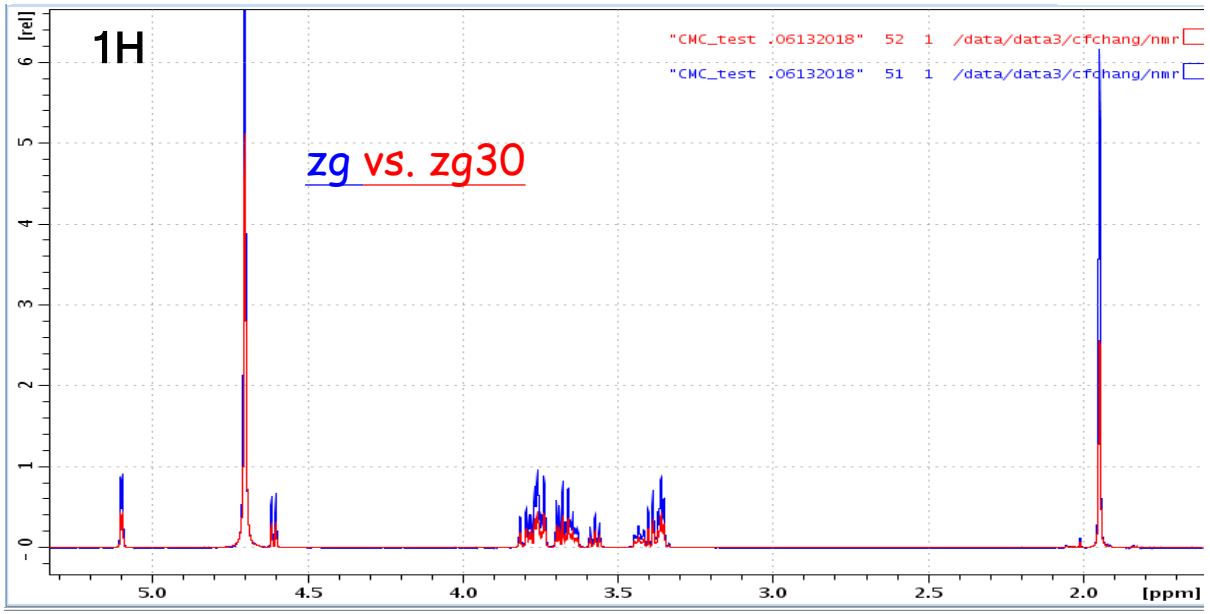
zg30

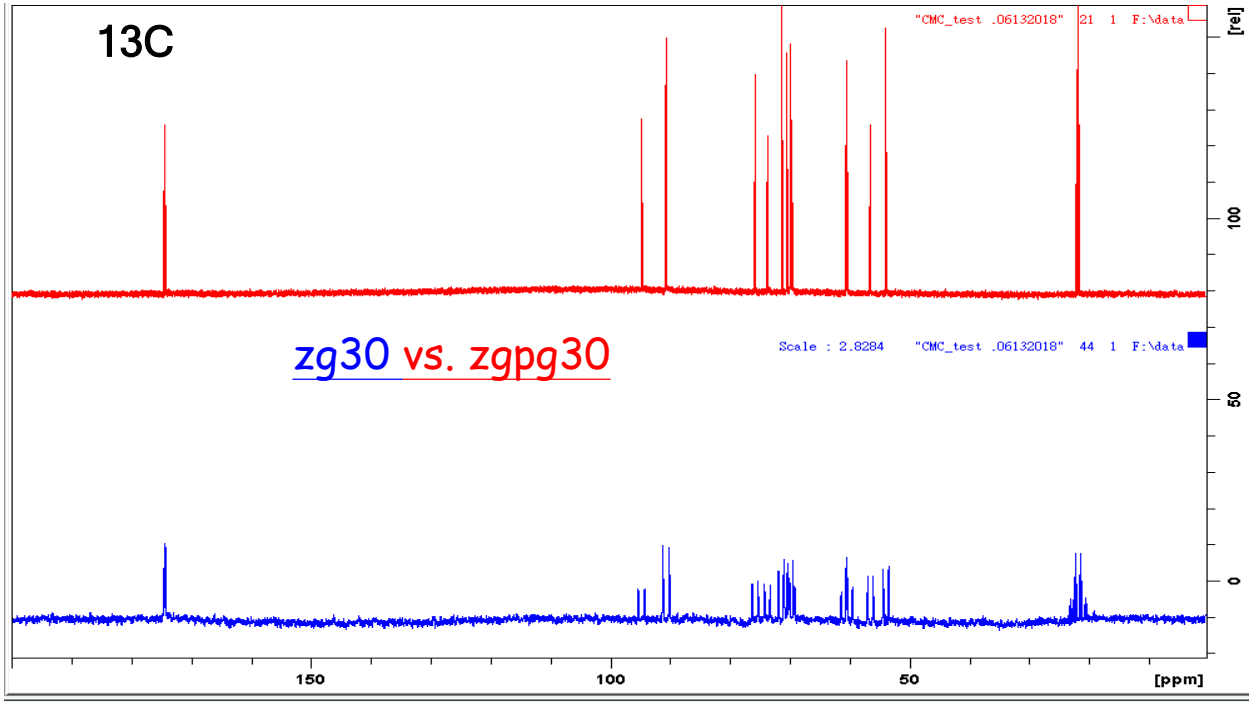


zgpg30 pl13 - pl12

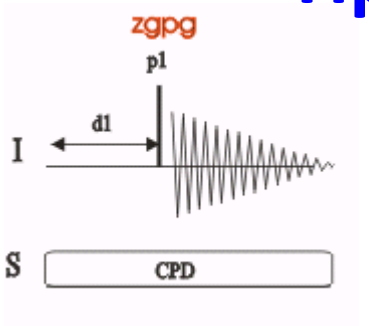
zgdc30



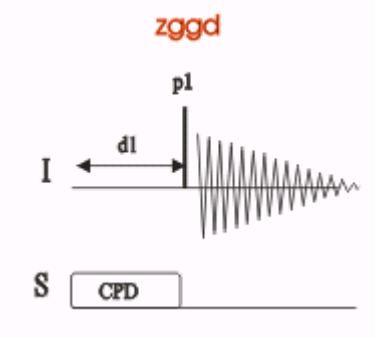




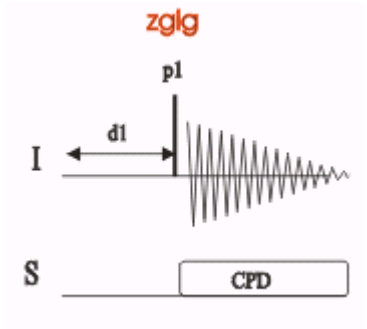
Tips on 1D 13C with 1H couple/decouple



Broad band decoupled methodology: Proton decoupling is applied during the acquisition/relaxation period. Resulting ^{1}H-decoupled ^{13}C spectrum .

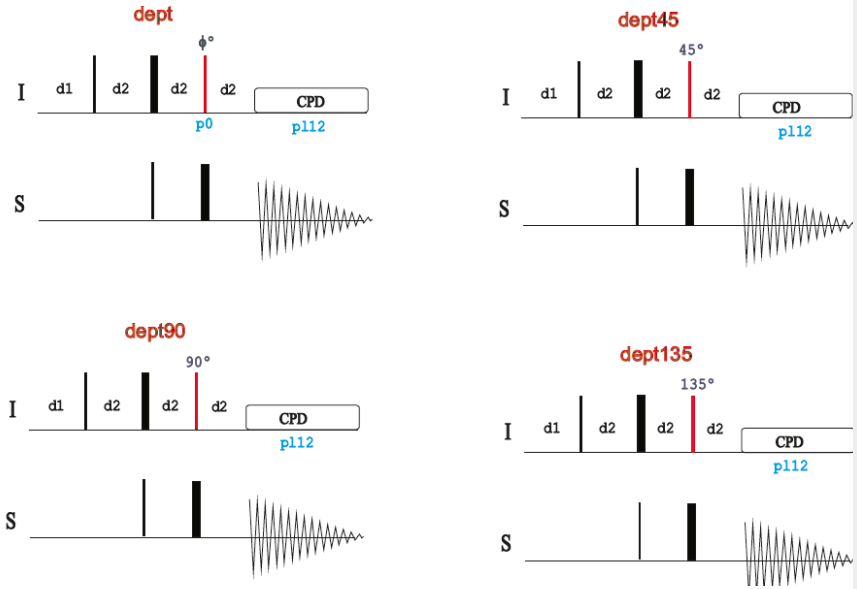


Gated decoupled methodology: Proton decoupling is only applied during the relaxation period. From the resulting ^{1}H-coupled ^{13}C spectrum carbon multiplicities, and direct and long range ^{1}H-^{13}C coupling constants can be determined.

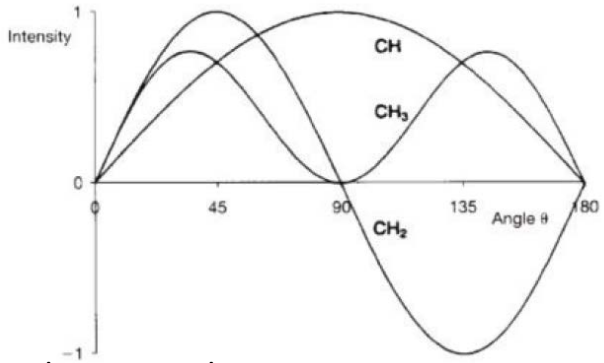


Inverse gated decoupled methodology: Proton decoupling is only applied during the acquisition period. In this case, no polarization transfer from ^{1}H to ^{13}C via NOE takes place and therefore, the resulting ^{1}H-decoupled ^{13}C spectrum can be used for quantitative measurements.

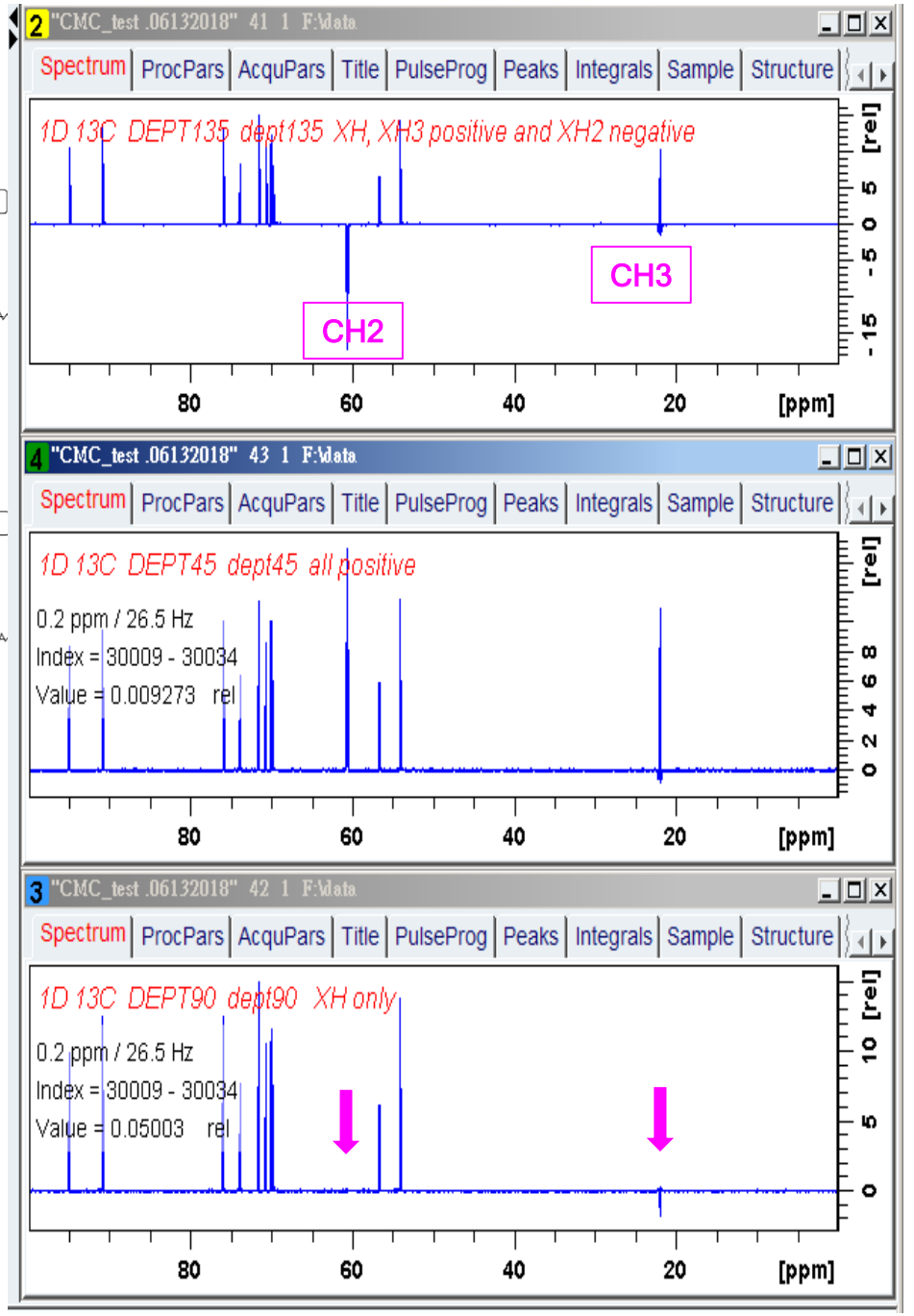
1D DEPT



The delay d2 is optimized to 1/2J(XH).

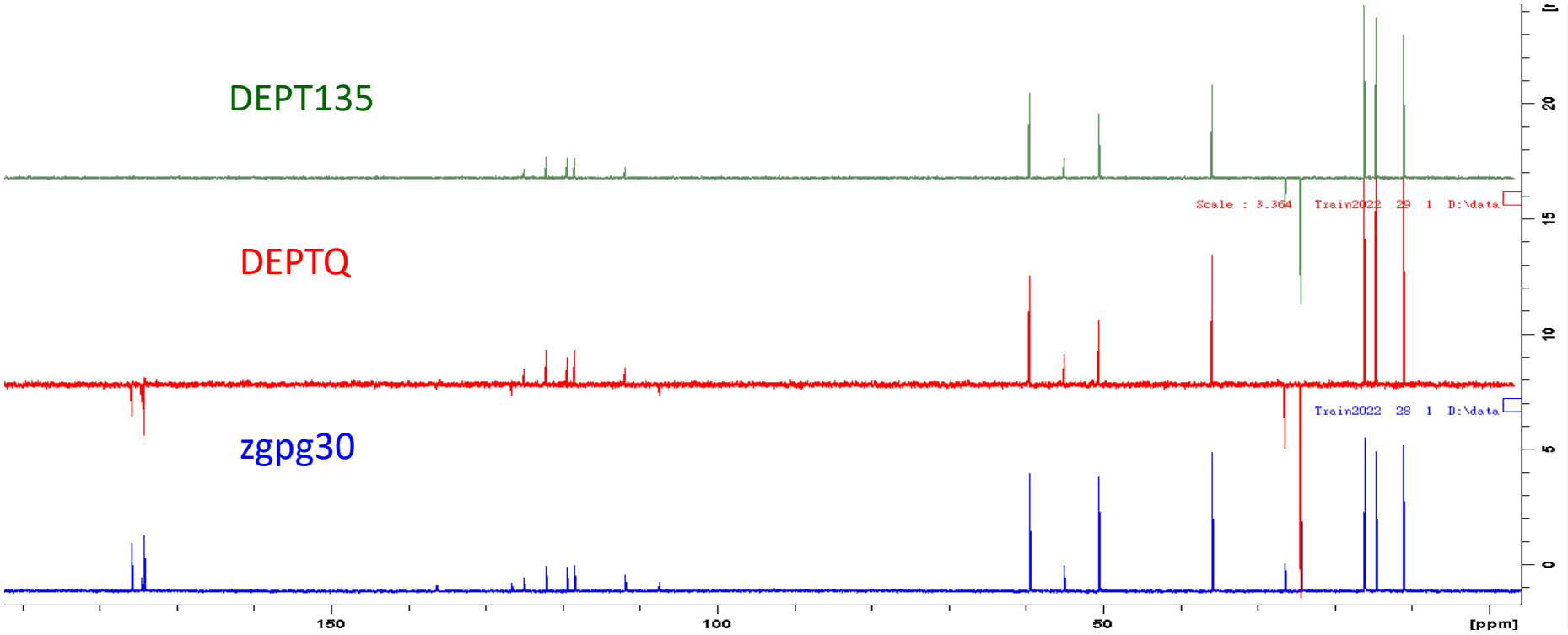


from Bruker Manual

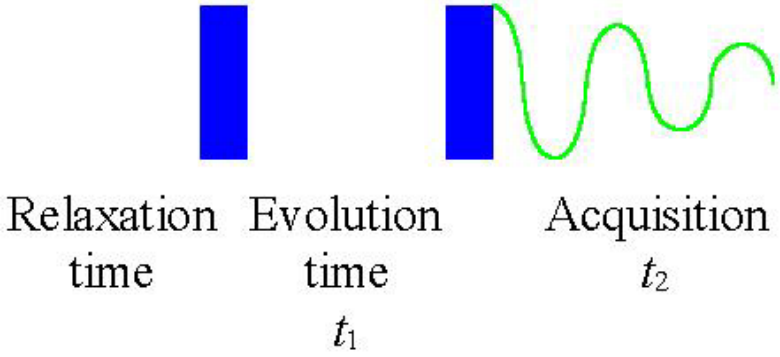


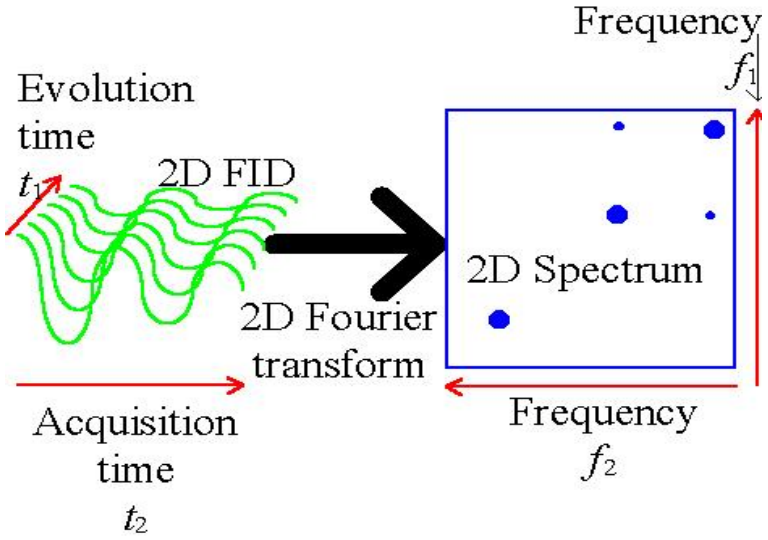
More for DEPT

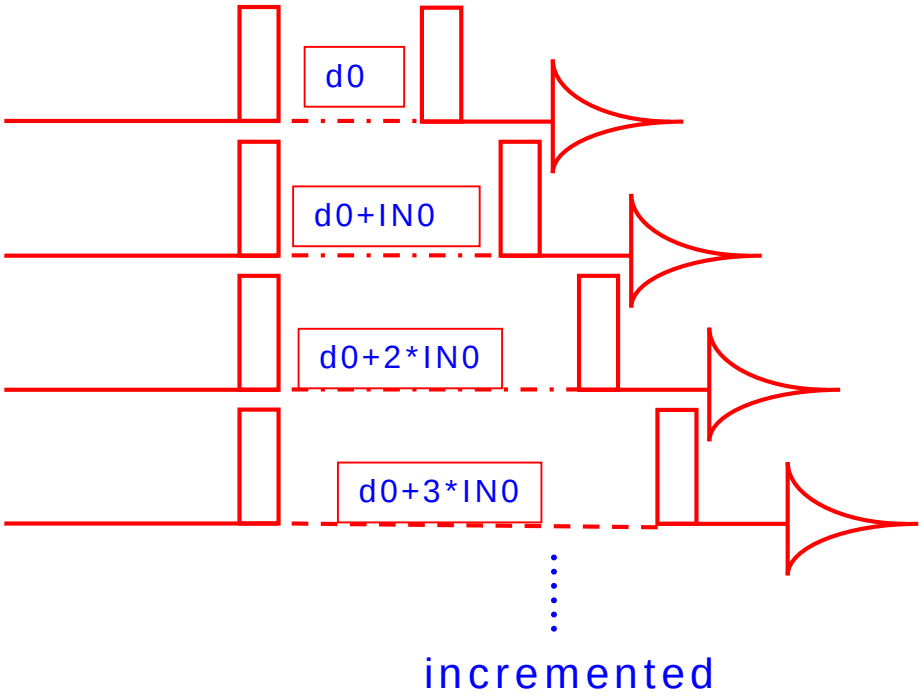
Expt	C	CH	CH2	CH3
DEPT45	NA	Positive	Positive	Positive
DEPT90	NA	Positive	NA	NA
DEPT135	NA	Positive	Negative	Positive
DEPTQ	Negative	Positive	Negative	Positive



2D NMR Experiment







FT

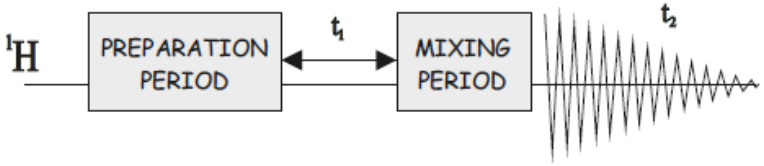
F (t1) → F (w1)

FT

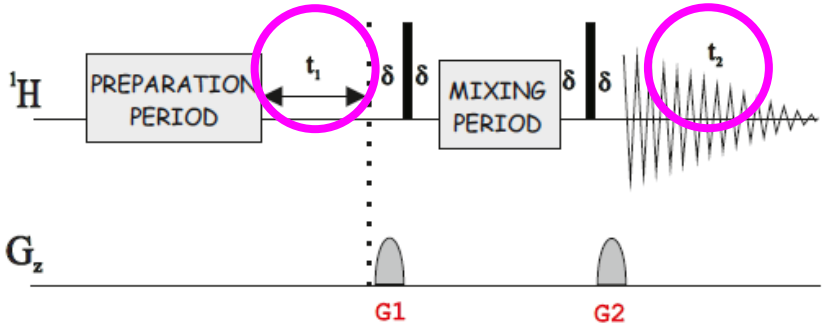
F (t2) → F(w2)

GENERAL 2D HOMONUCLEAR SCHEMES

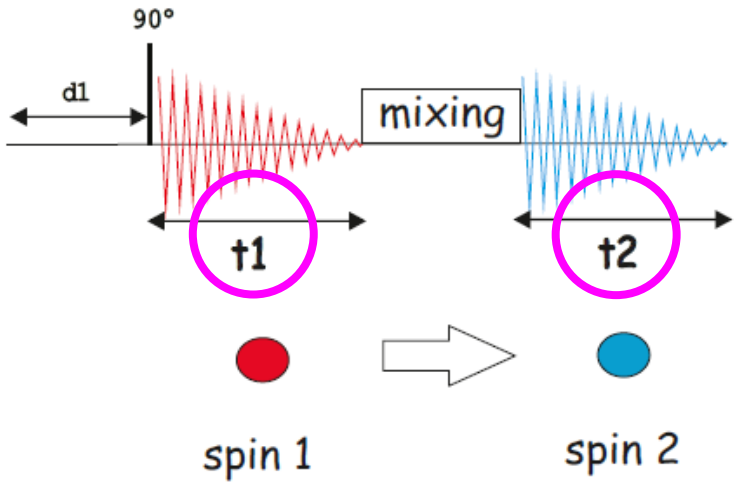
1. Phase-Cycled homonuclear



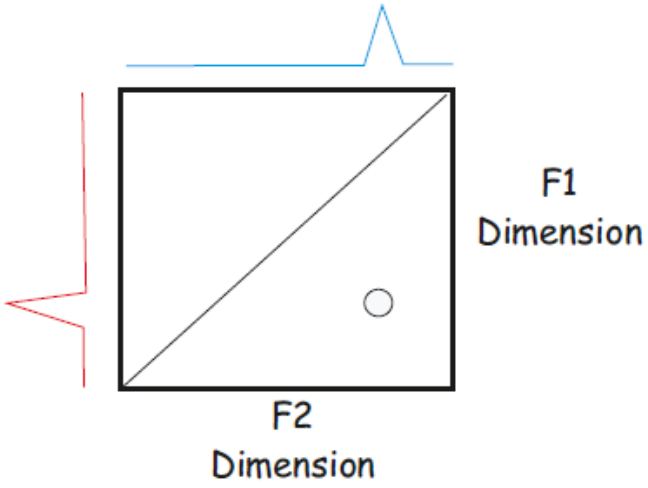
2. Gradient-Enhanced homonuclear



Imagine an experiment in which the information of spin 1 is transferred to spin 2

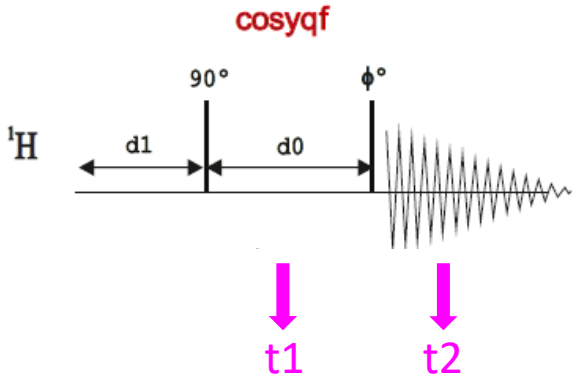


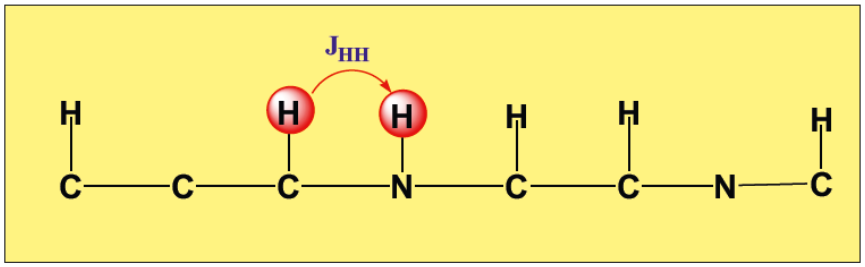
The resulting 2Dmap looks like this

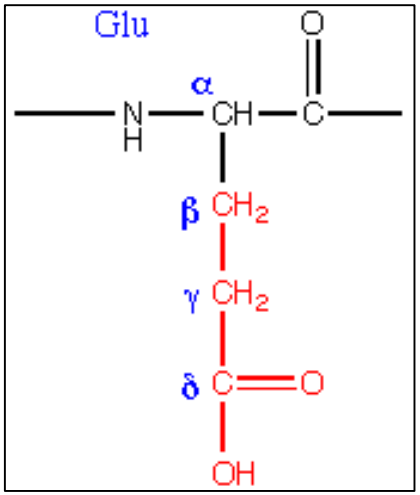


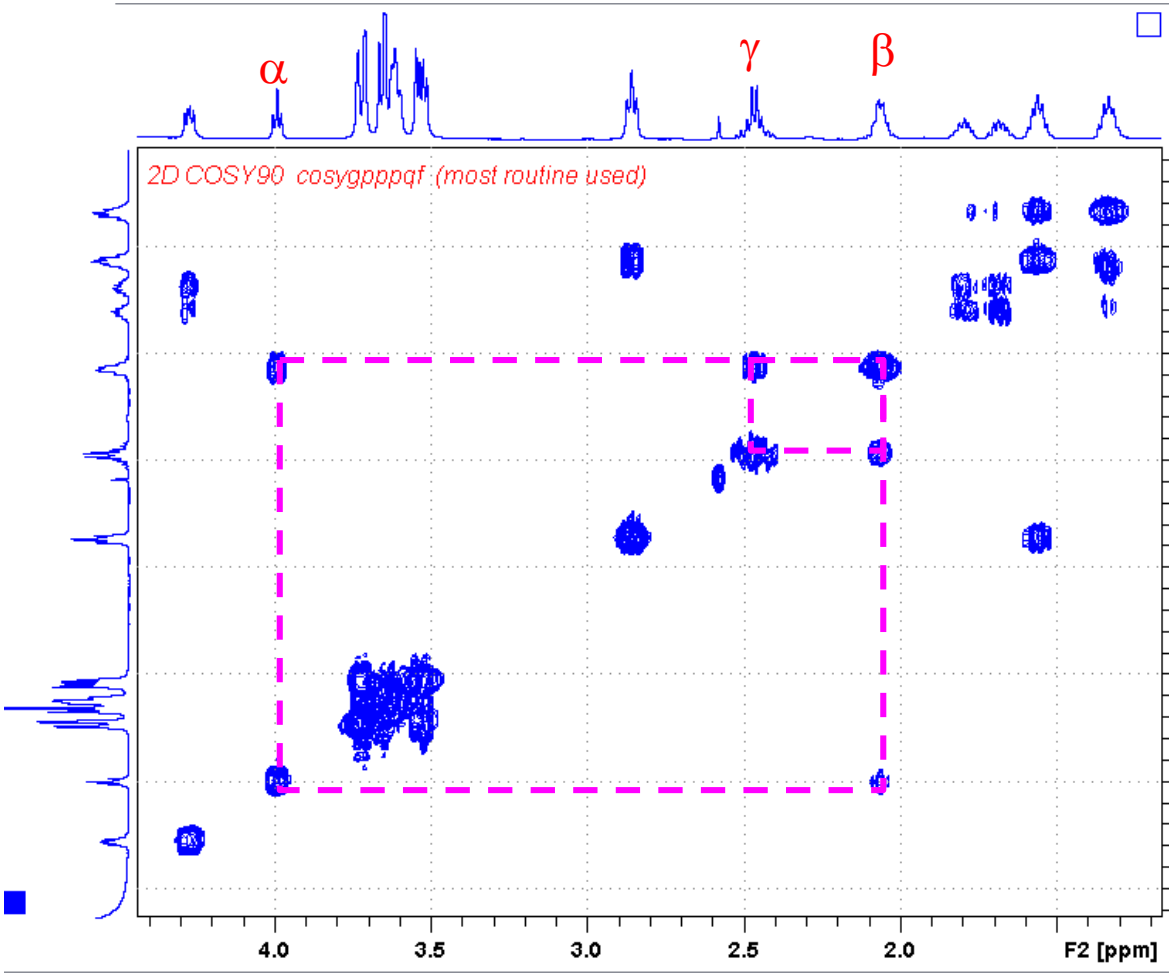
from Bruker Manual

2D homo nuclear COSY

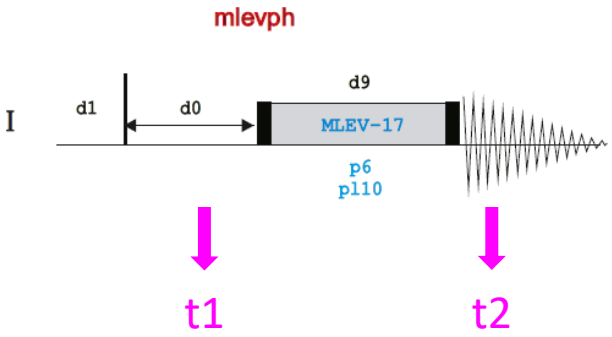


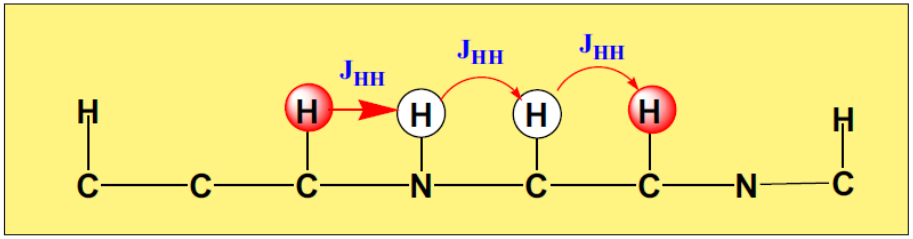


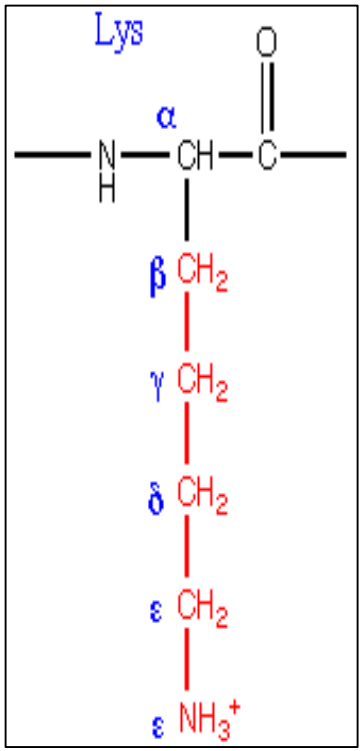


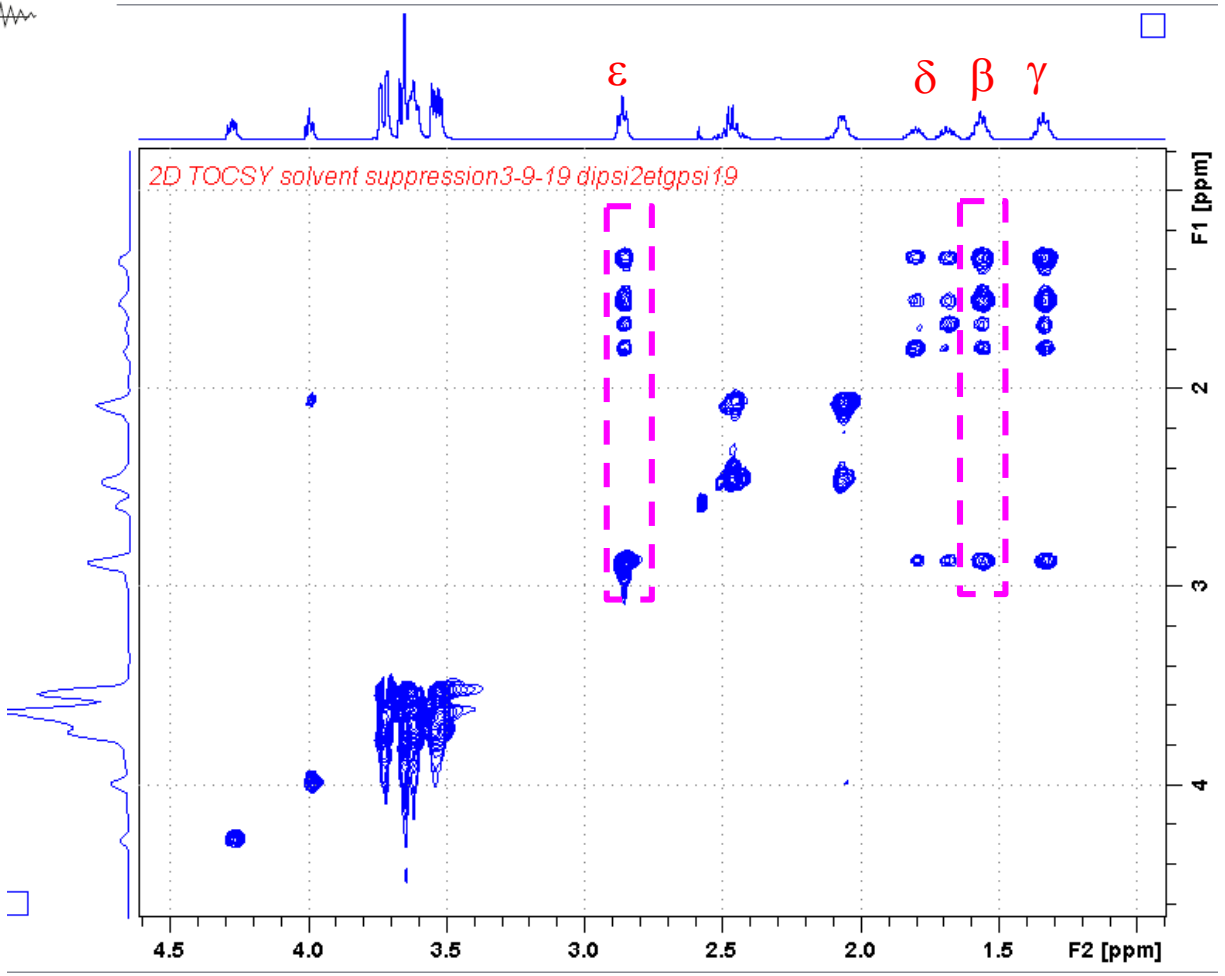


2D homo nuclear TOCSY

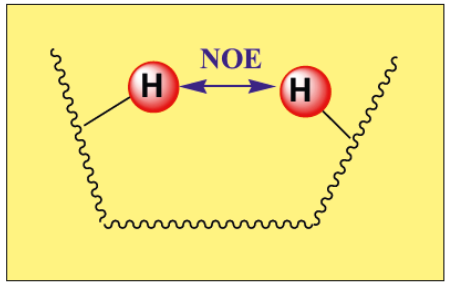






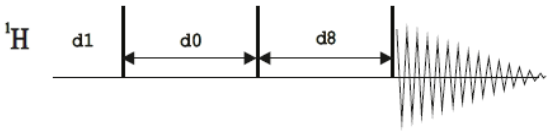


2D homo nuclear NOESY or ROESY

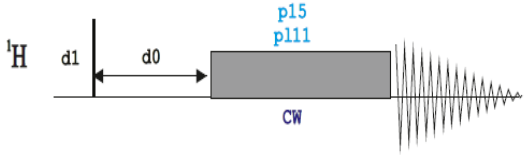


noesyph

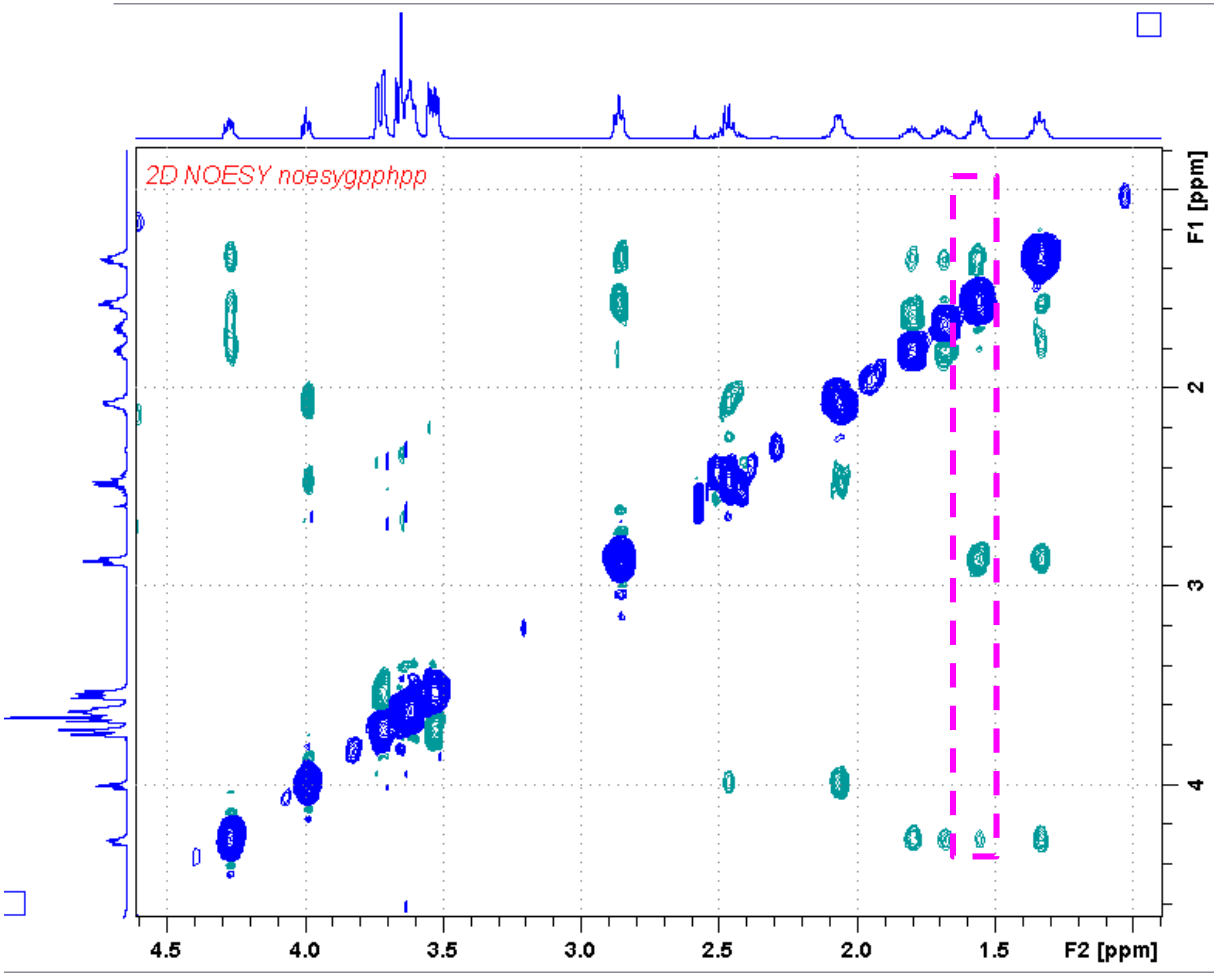
noesyphrv



roesyph



t1 t2



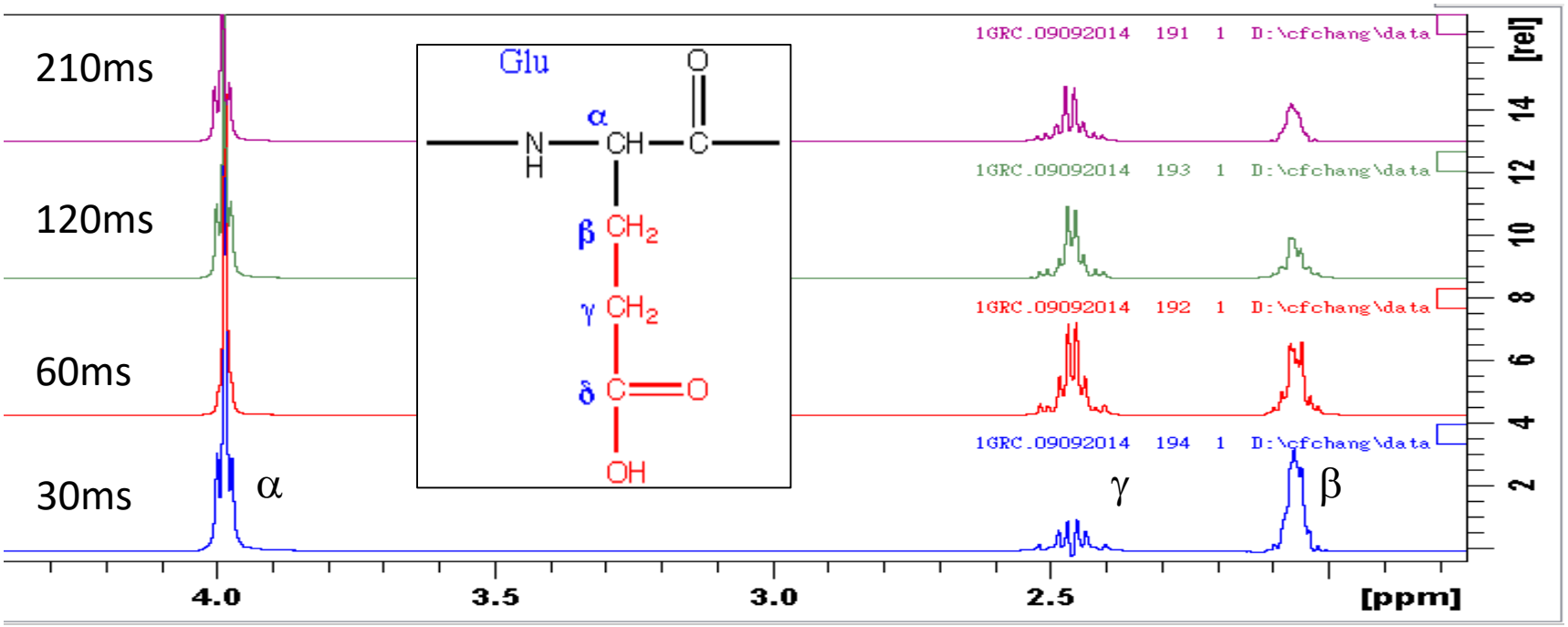
Tips on 2D TOCSY & NOESY/ROESY

Experiment	Mixing Time	Note
TOCSY	20-100ms	Longer mixing time, more distant protons can be observed , but… adjacent one might become weaker
NOESY / ROESY	Depends on MW	Need to pay attention on spin diffusion

Molecular Weight	Experiment	Mixing Time	Note
MW<600	NOESY	>500ms (ex:500ms)	Diagonal peaks + Crossed peaks -
600<MW<1500	ROESY	100ms~800ms (ex: 250ms)	All positive
Mw>1500	NOESY (ROESY suffer less spin diffusion but less sensitive too)	50ms~200ms (ex: 120ms)	All positive

Tips on 2D TOCSY & NOESY/ROESY

Experiment	Mixing Time	Note
TOCSY	D9=20-100ms	Longer mixing time, more distant protons can be observed , but… adjacent one might become weaker

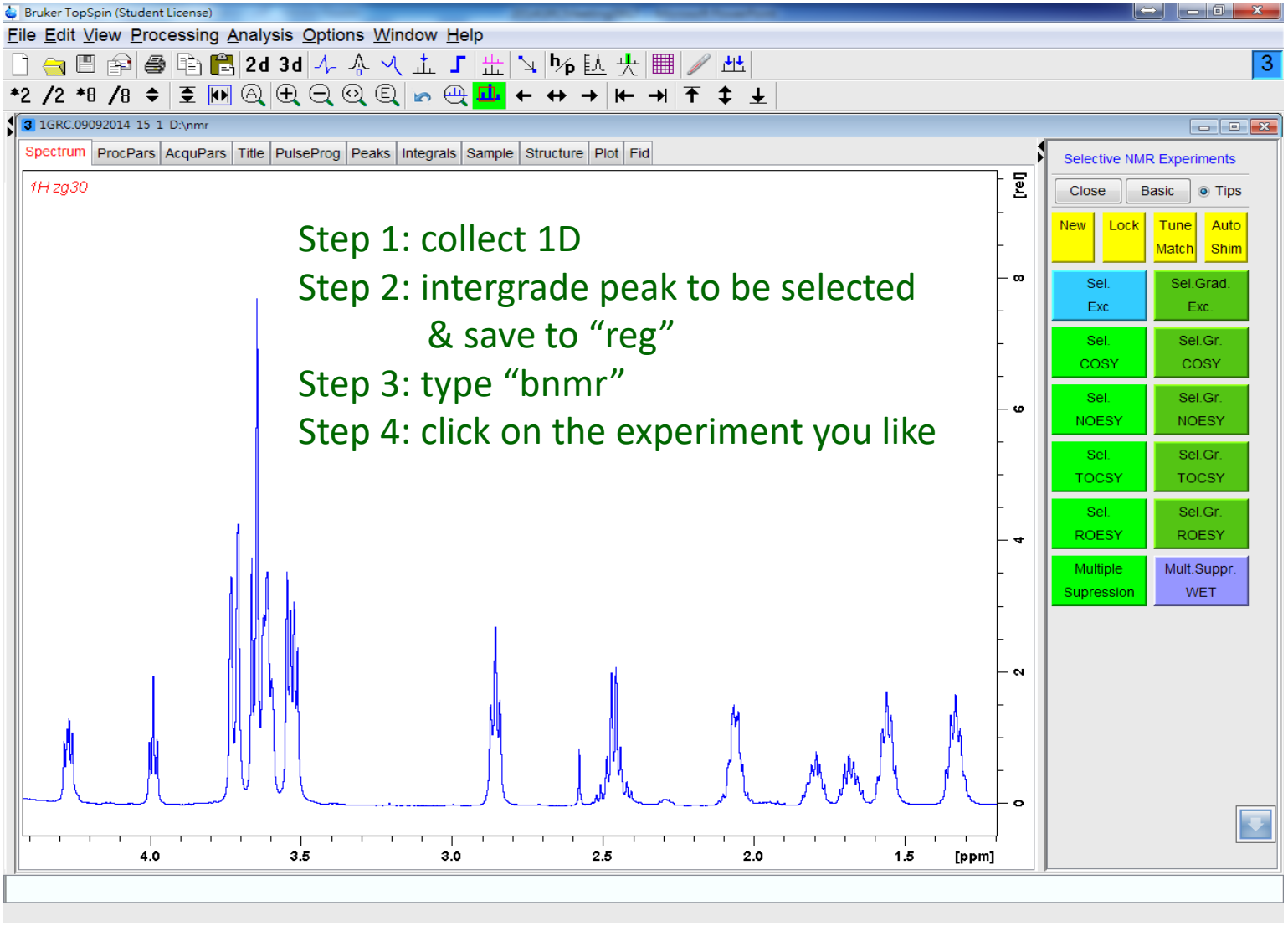


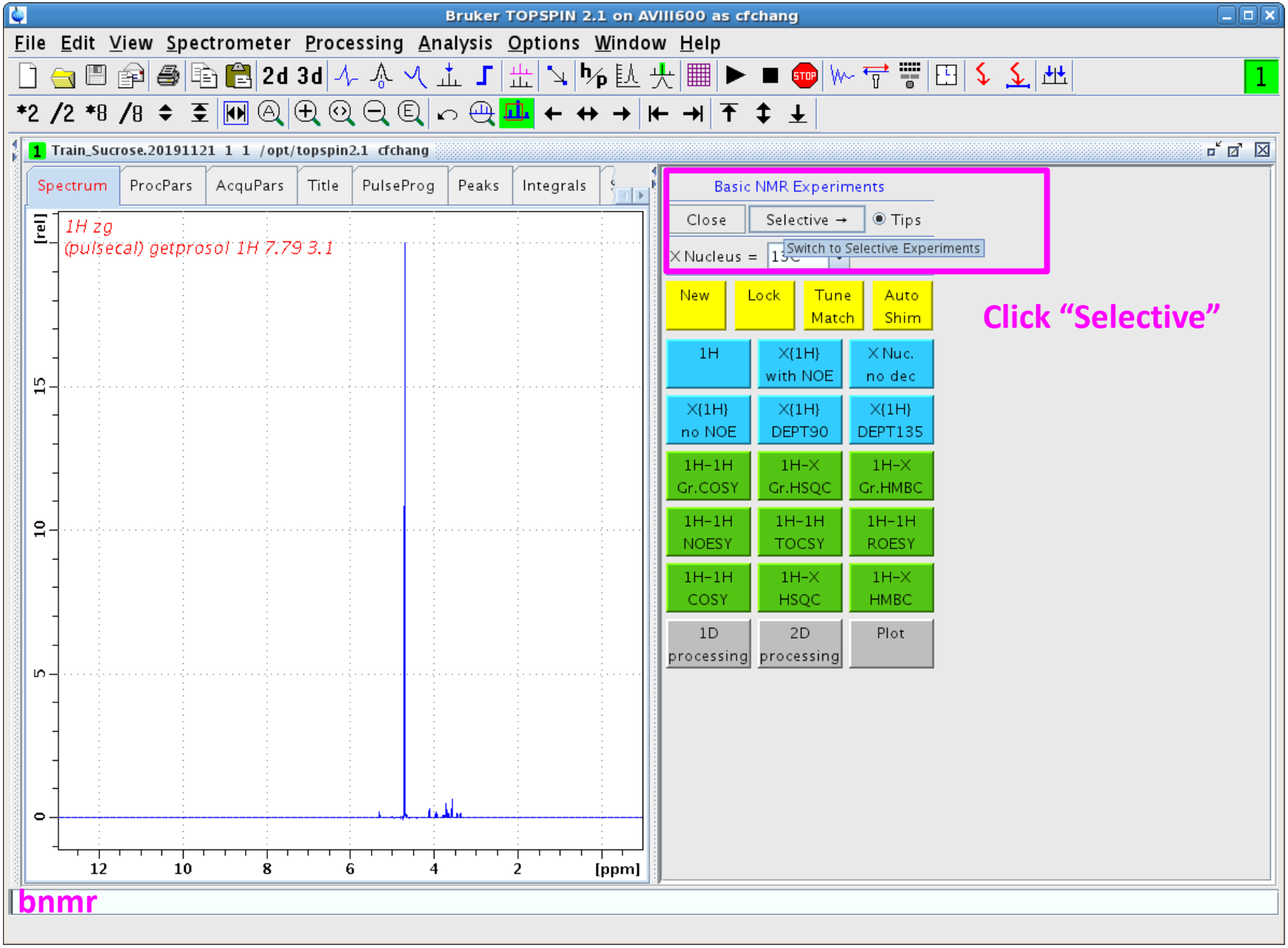
2D 1H-1H Experiments

Parameter Set	Experiment Details	Note
2D_COSY_cosygpppqf	1H-1H COSY	Most useful
2D_COSY-sol_cosygpprqf	1H-1H COSY with solvent supression	
2D_COSY-sol_cosyprqf	1H-1H COSY with solvent supression	
2D_COSY45_cosyqf45	1H-1H COSY	
2D_COSY-dec13C_cosydcgpqf	1H-1H COSY with 13C decoupled	
2D_COSY-DQF-sol_cosydfgpph19	1H-1H COSY with solvent supression	DQF
2D_COSY-DQF_cosygpmfph	1H-1H COSY	DQF
2D_TOCSY_dipsi2etgpsi	1H-1H TOCSY	Most useful
2D_TOCSY-sol_dipsi2etgpsi19	1H-1H TOCSY with solvent supression	
2D_TOCSY-sol_dipsi2phpr	1H-1H TOCSY with solvent supression	
2D_NOESY_noesygpphpp	1H-1H NOESY	Most useful
2D_NOESY-sol_noesygpph19	1H-1H NOESY with solvent supression	
2D_NOESY-sol_noesygpphpr	1H-1H NOESY with solvent supression	
2D_NOESY-sol_noesyphpr	1H-1H NOESY with solvent supression	
2D_ROESY_roesyetgp	1H-1H ROESY	Most useful
2D_ROESY_roesyphpp	1H-1H ROESY	
2D_ROESY-sol_roesygpph19.2	1H-1H ROESY with solvent supression	
2D_ROESY-sol_roesyphpr	1H-1H ROESY with solvent supression	

1D Selected Excitation Experiments

Use button NMR

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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
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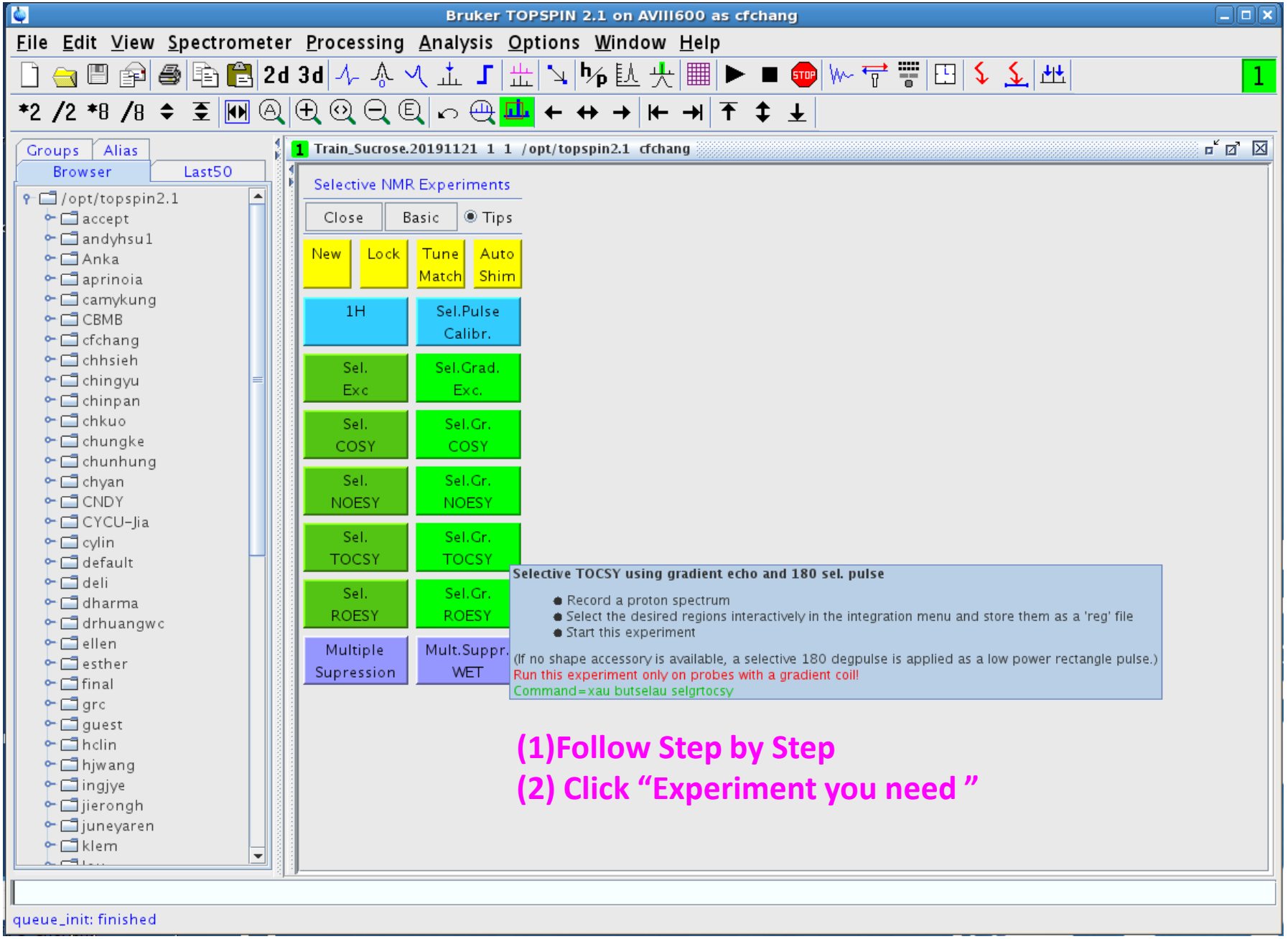
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CYCU-Jia
cylin
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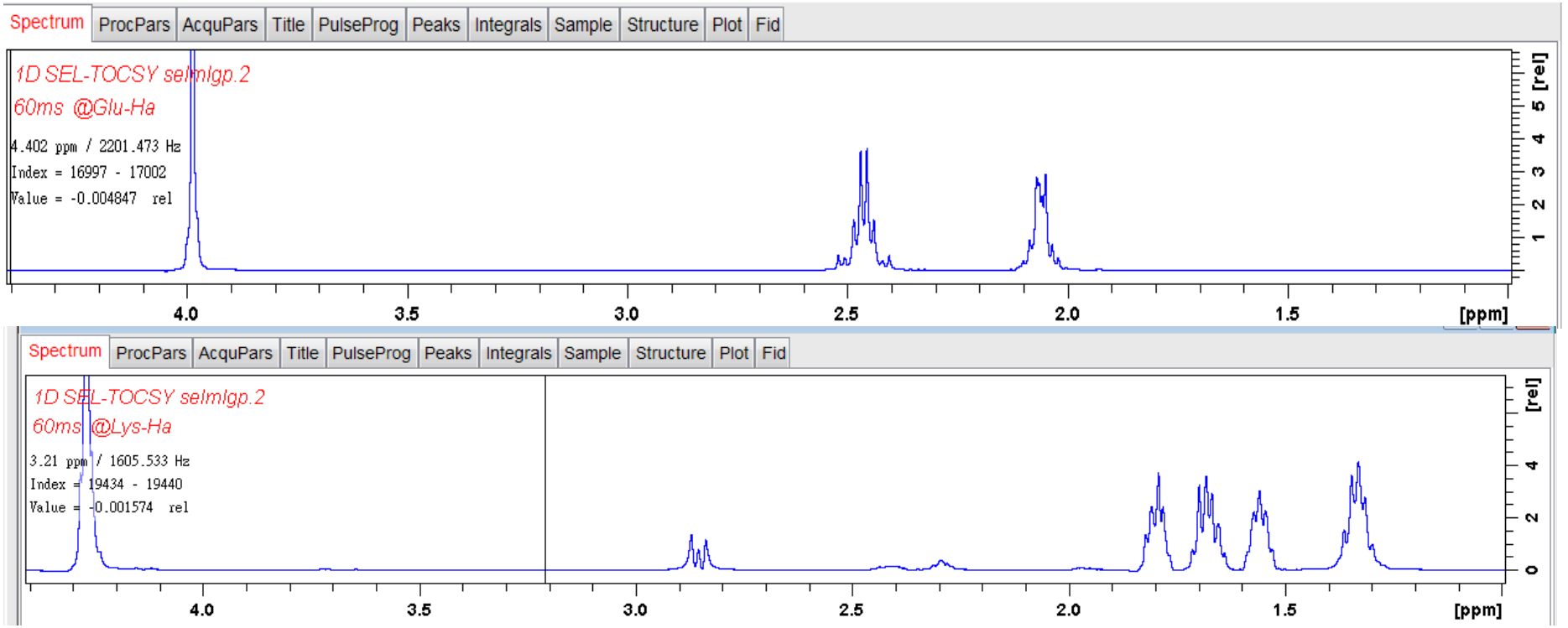
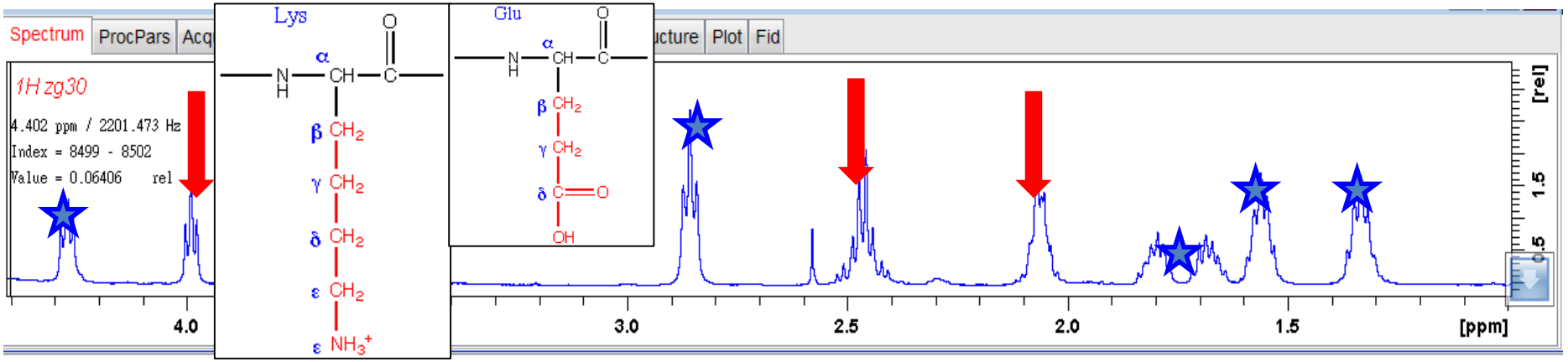
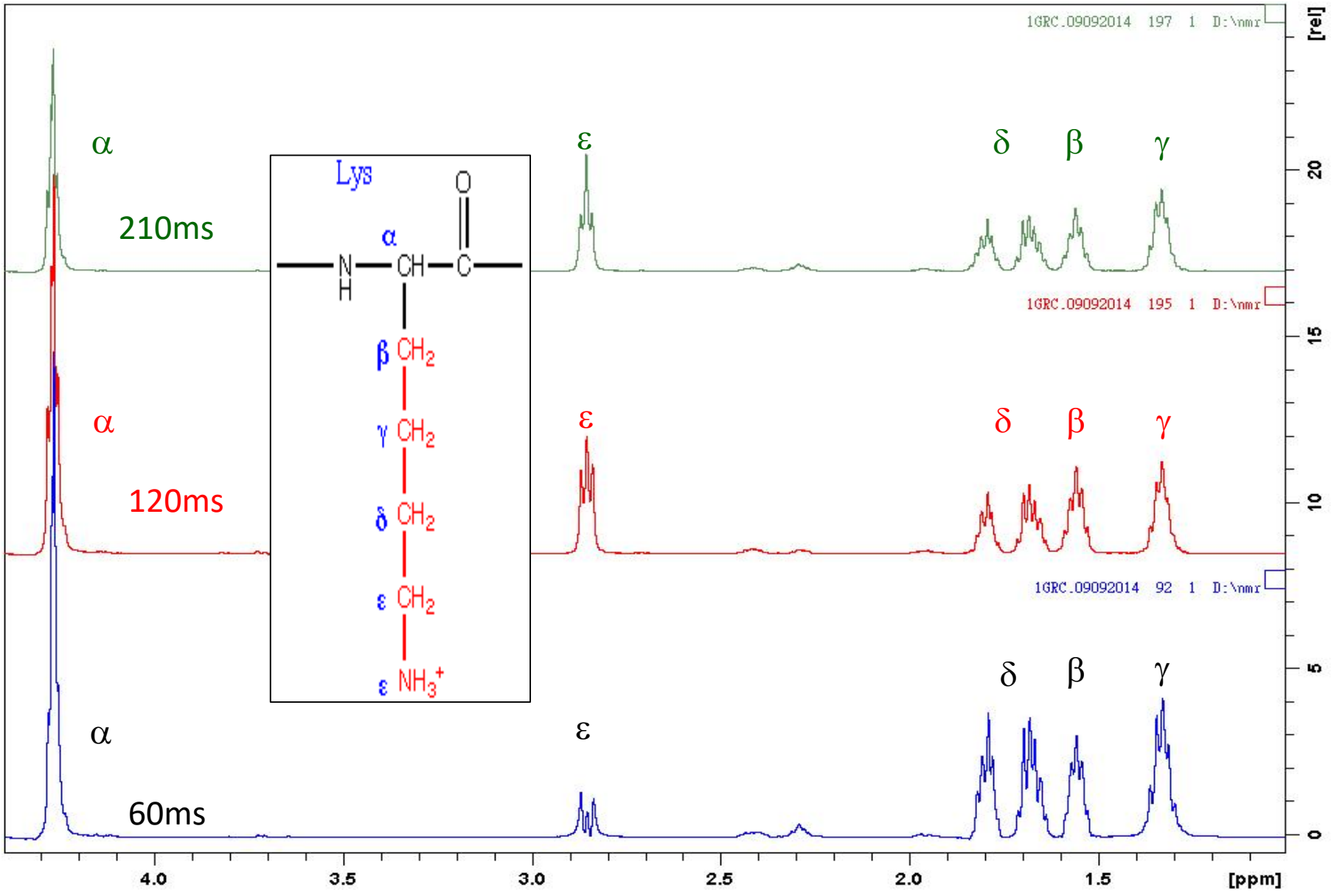
Sel. COSY
Sel.Gr. COSY
Sel. NOESY
Sel.Gr. NOESY
Sel. TOCSY
Sel.Gr. TOCSY
Sel. ROESY
Sel.Gr. ROESY
Multiple Supression
Mult.Suppr. WET
Selective TOCSY using gradient echo and 180 sel. pulse
Record a proton spectrum
Select the desired regions interactively in the integration menu and store them as a 'reg' 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 selmlgp.2

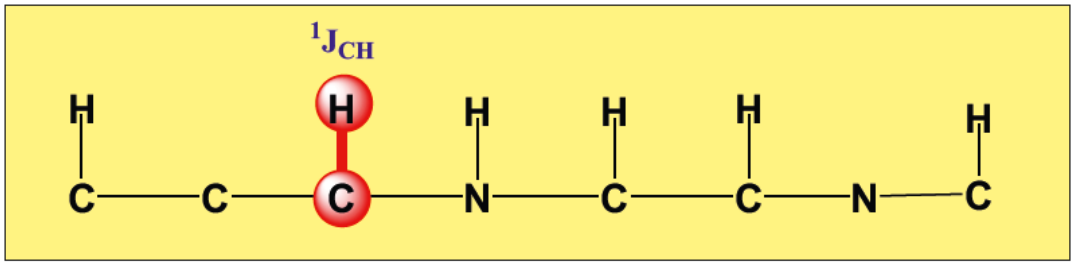


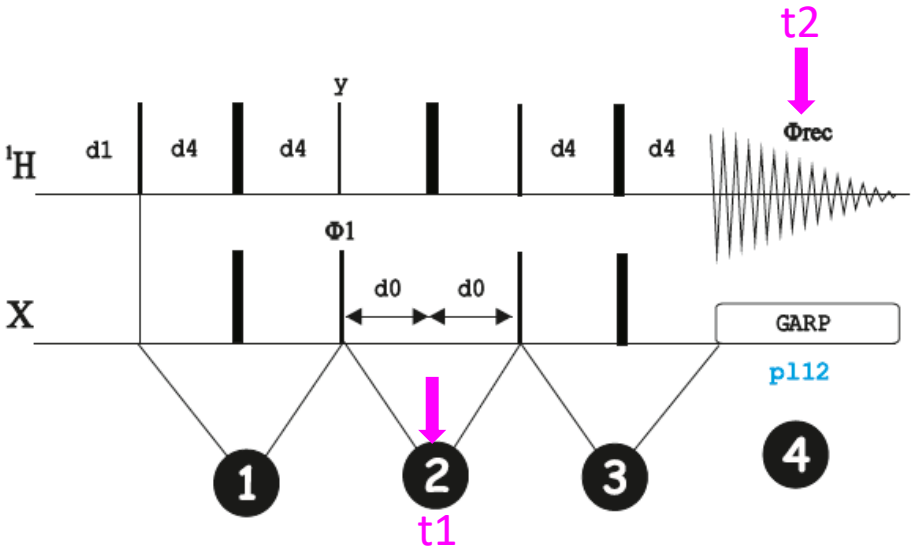


Tips on selected TOCSY : optimize mixing time



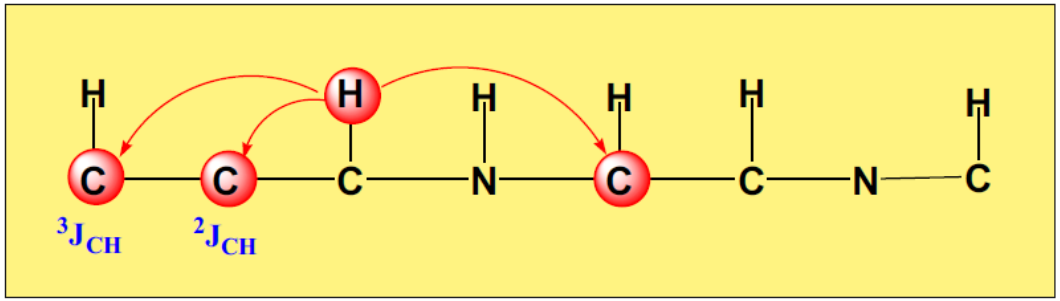
2D hetero nuclear HSQC or HMQC

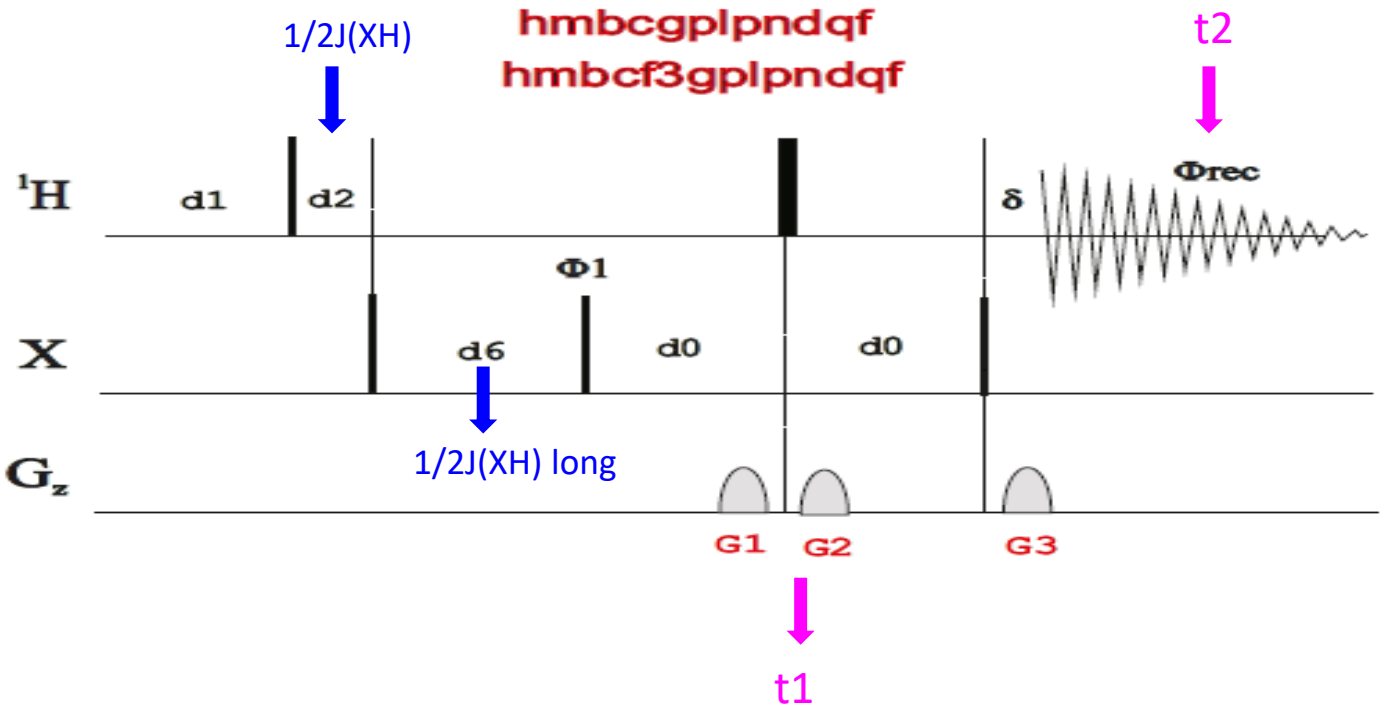




1. 1H-to-X INEPT transfer optimized to 1/4J(XH)
2. δ(X) evolution and J(CH) refocusing during variable d0 period
3. X-to-1H retro-INEPT transfer optimized to 1/4J(XH)
4. 1H detection with optional X-decoupling

2D hetero nuclear HMBC





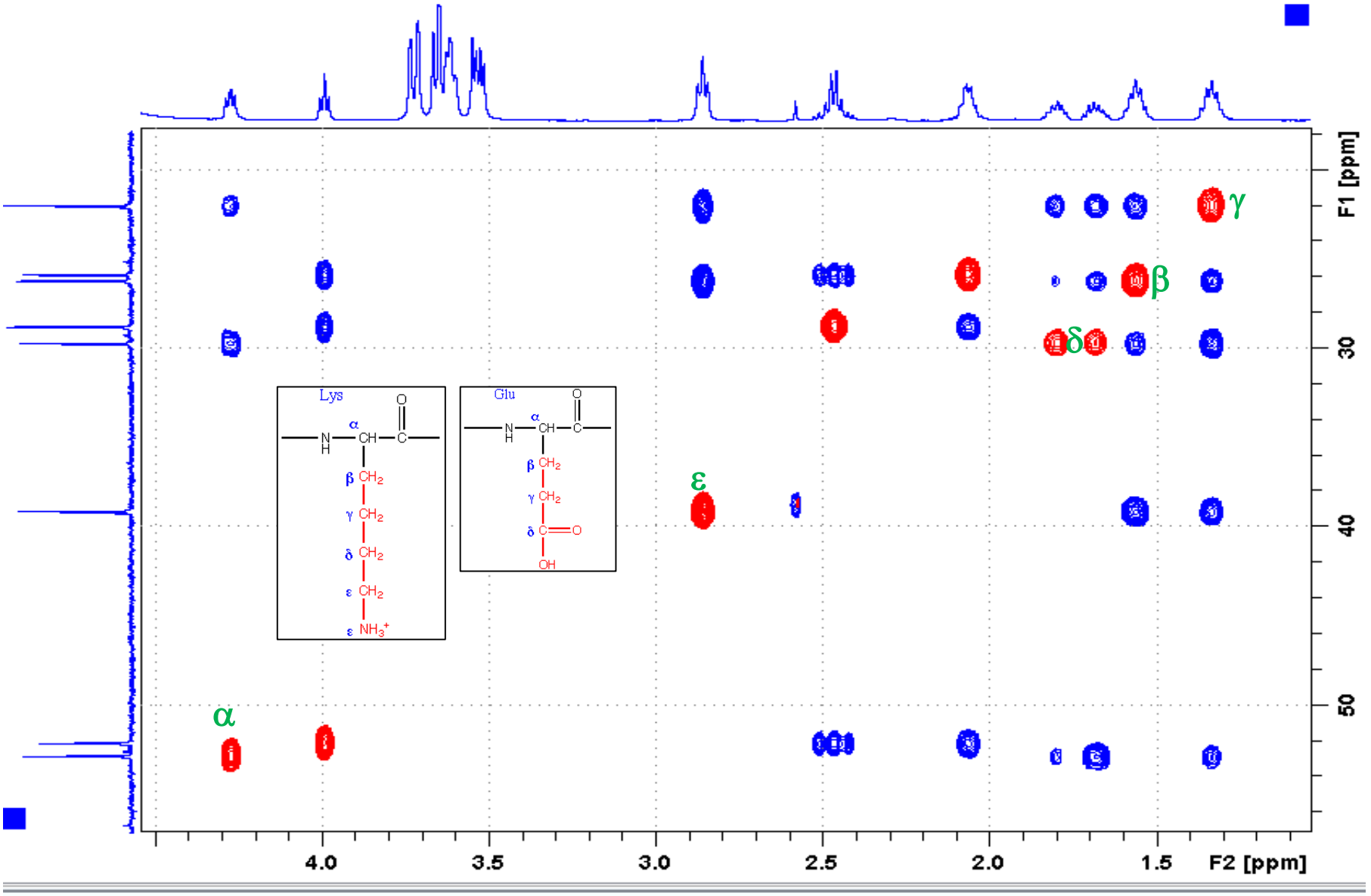
cnst2: = J(XH)

cnst13: = J(XH) long range

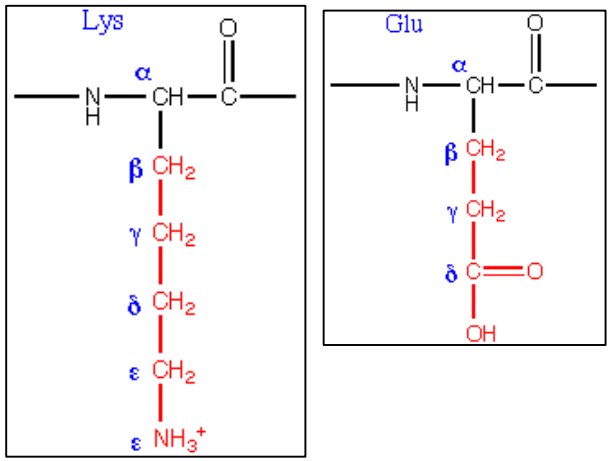
d2=1s/(cnst2*2)"

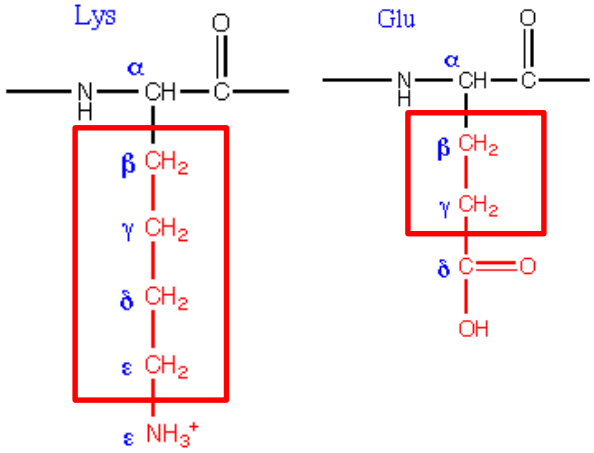
d6=1s/(cnst13*2)"

2D hetero nuclear
HMBC vs. HSQC

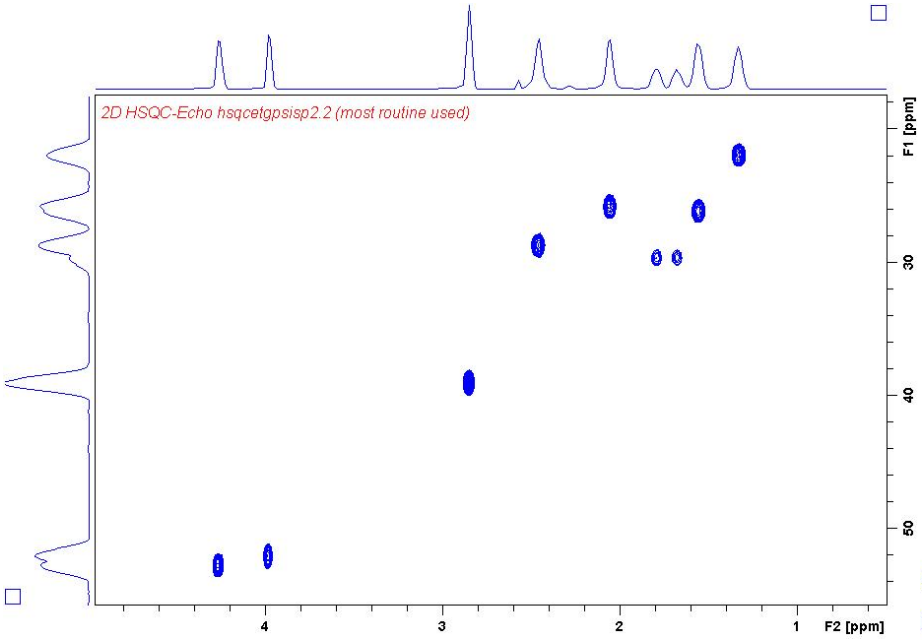


Example on different version HSQC

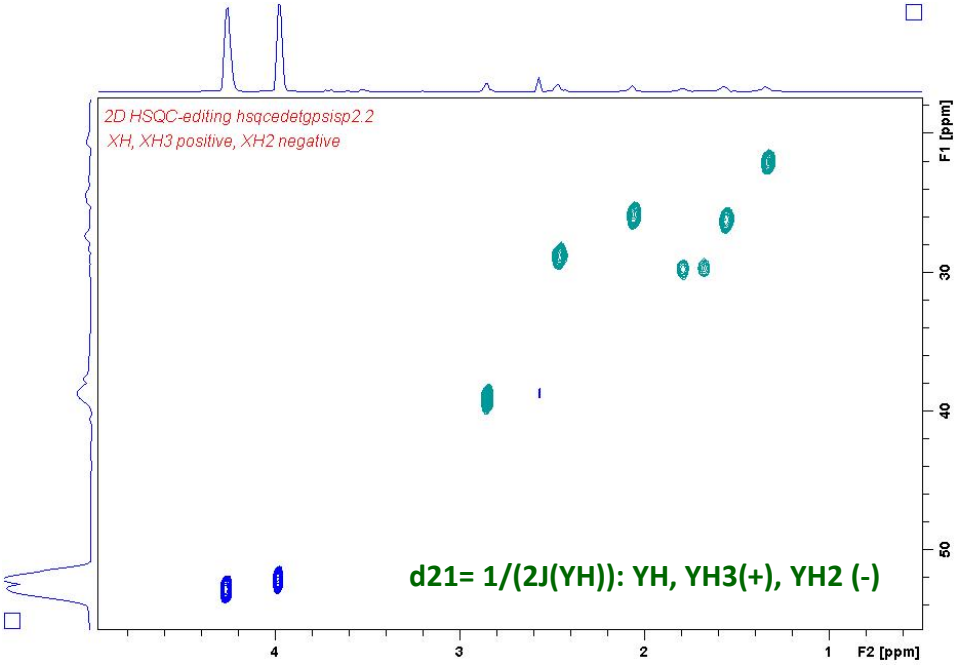




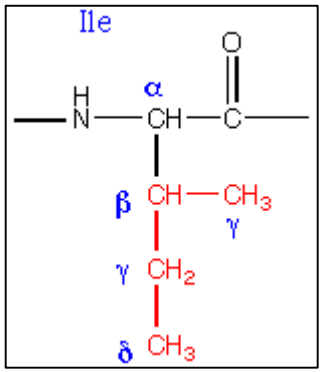
1GRC_2D_HSQC_hsqcetgpsisp2.2

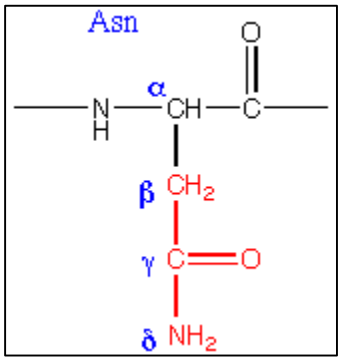


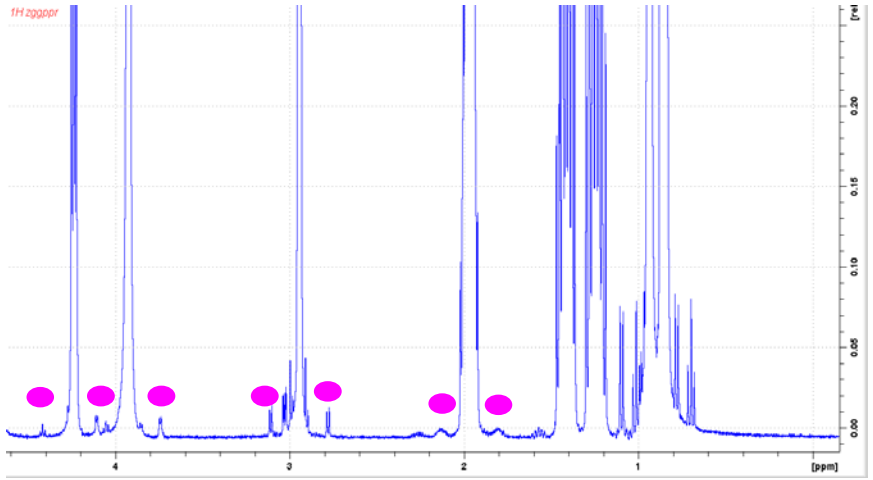
1GRC_2D_HSQC-editing_hsqcedetgpsisp2.2



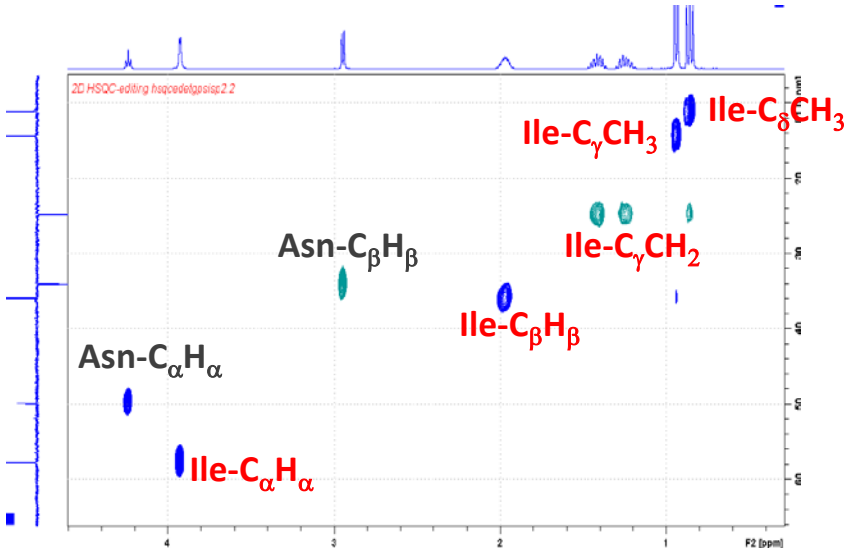
Example on different version HSQC



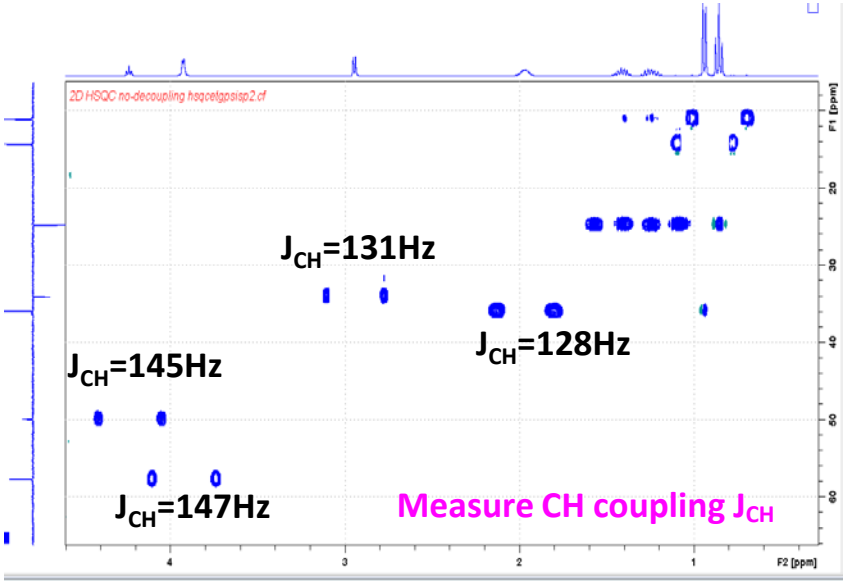




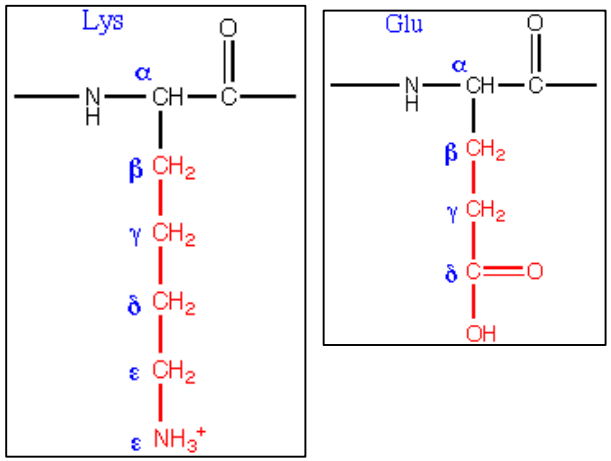
1GRC_2D_HSQC-editing_hsqcedetgpsisp2.2

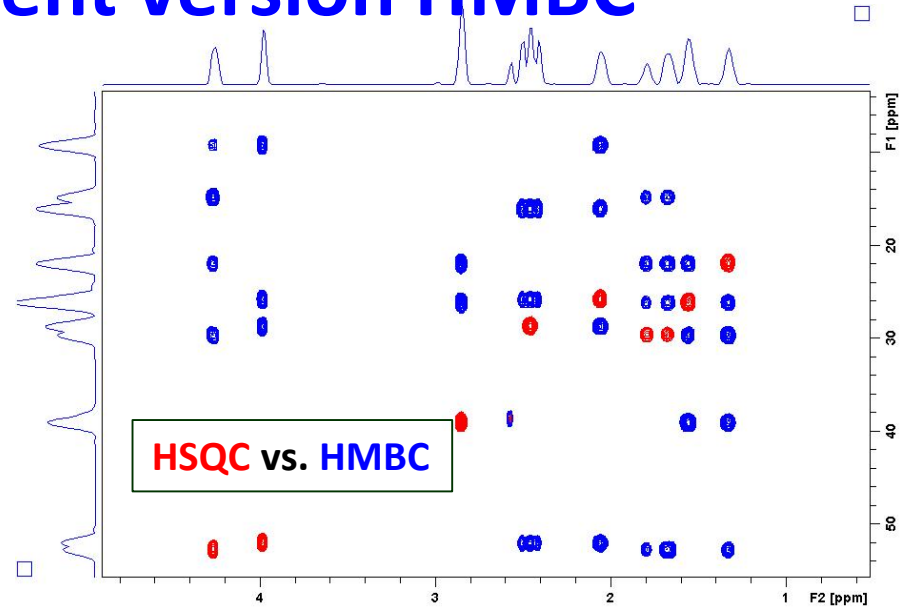


1GRC_2D_HSQC_nodec_hsqcetgpsisp.cf

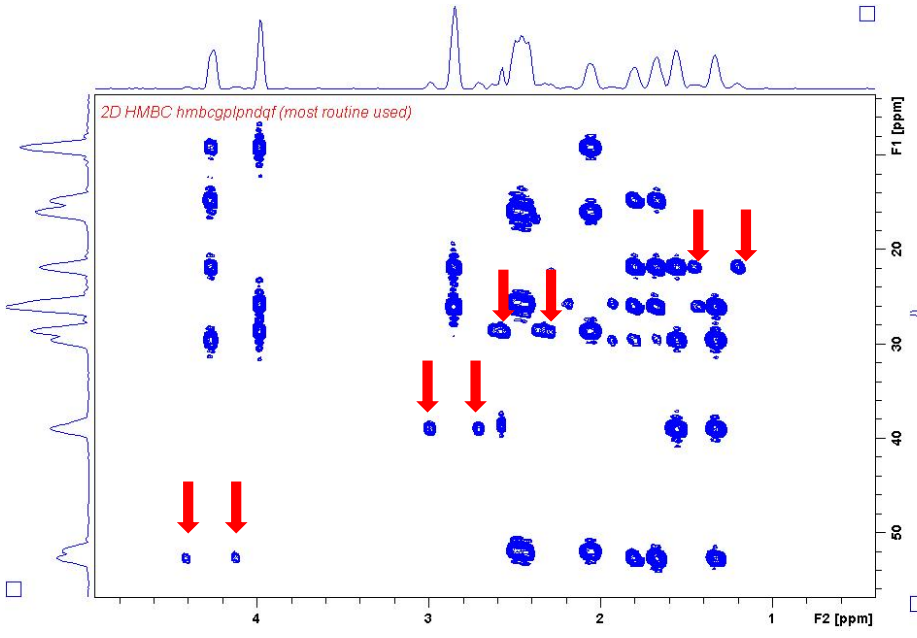


Example on different version HMBC

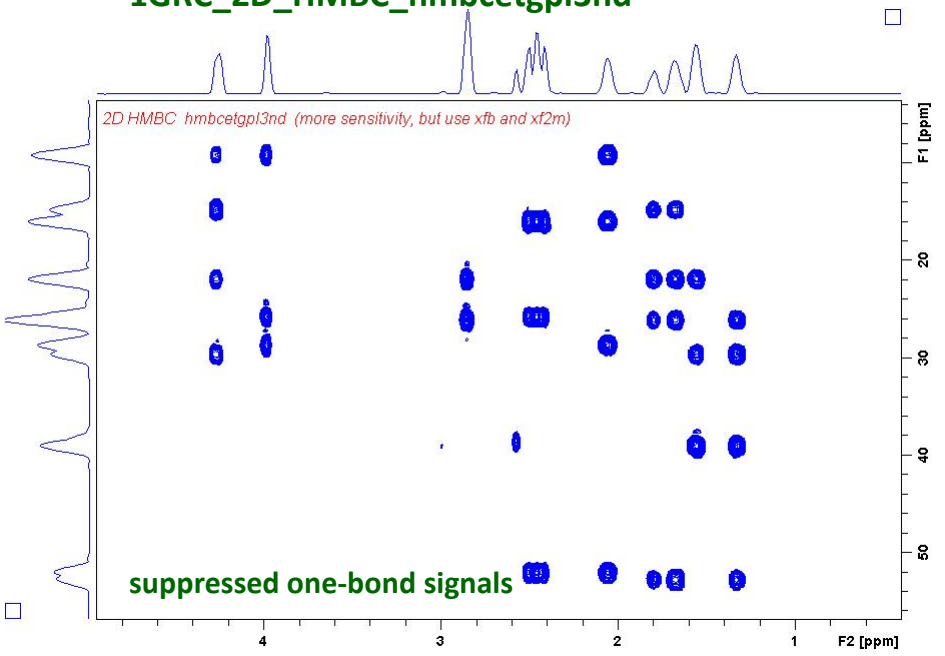




1GRC_2D_HMBC_hmbcgplpndqf



1GRC_2D_HMBC_hmbcetgpl3nd



2D 1H-13C Experiments

Parameter Set	Experiment Details	Note
2D_HSQC_hsqcetgpsisp2.2	1H-13C HSQC	Most useful
2D_HSQC-editing_hsqcedetgpsisp2.2	1H-13C edited HSQC	d21= 1/(2J(YH)): YH, YH3(+), YH2 (-) d21= 1/(4J(YH)): YH2 only
2D_HSQC-nodec_hsqcetgpsisp.cf	Determine XH coupling	
2D_HSQC-shsqcetgpsisp2.2cas	1H-13C HSQC	(shape pulse)
2D_HSQC-sol-hsqcetgpprsisp2.2	1H-13C HSQC with solvent suppression	
2D_HMQC-hmqcetgpsi.2	1H-13C HMQC	
2D_HMQC-hmqcgpqf	1H-13C HMQC	
2D_HMBC_hmbcgplpndqf	1H-13C HMBC	Most useful
2D_HMBC_hmbcetgpl3nd	1H-13C HMBC J-filter to suppressed one-bond	Good for “clean” spectrum
2D_HMBC-CIGAR_hmbcacgplpndqf.2	1H-13C HMBC optimized for long range signals	