Basic Operation for NMR Systems in Core Facility

Basic NMR Concepts & Experimental Set Up

by

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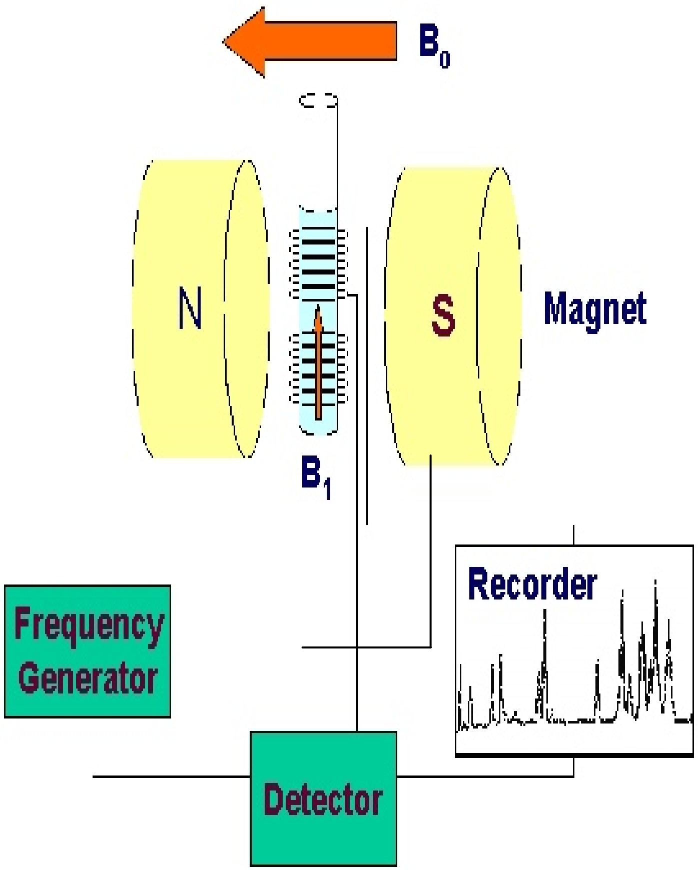
NMR Spectroscopy

Where is it?

1nm 10 10^2 10^3 10^4 10^5 10^6 10^7

(the wave)	X-ray	UV/VIS	Infrared	Microwave	Radio Frequency
(the transition)	Electronic Transition		Vibration	Rotation	Nuclear
(spectrometer)	**X-ray**	**UV/VIS**	**Infrared/Raman**		**NMR**
			Fluorescence		

Nuclear Magnetic Resonance Spectrometer

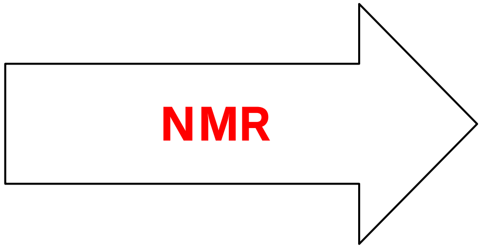


B_0: 光譜儀之磁場強度

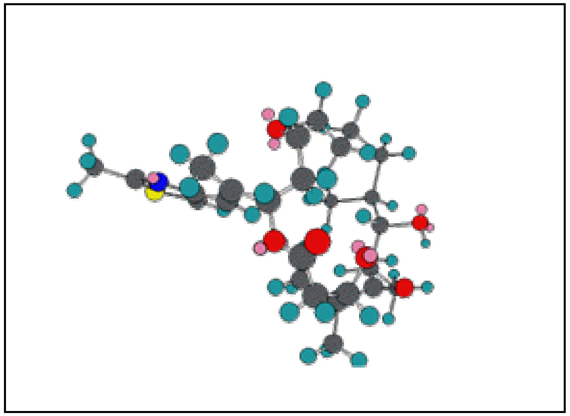
B_1: 外加小磁場 (來自樣品周圍之線圈)

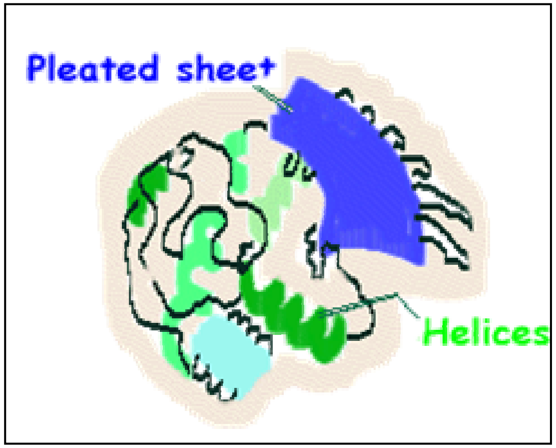
The problem the we want to solve

What we "really" see



What we want to "see"



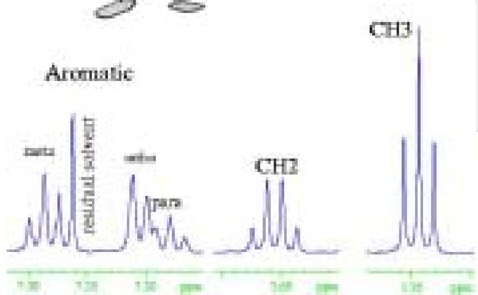


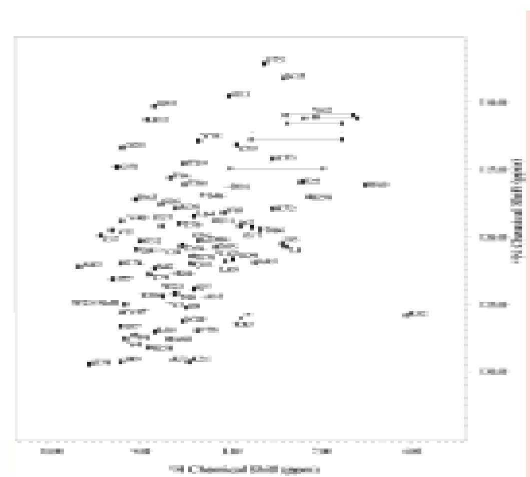
Steps for NMR Experiment

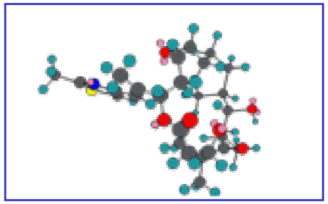
取得樣品 → 取得ＮＭＲ圖譜 → 分析圖譜結果

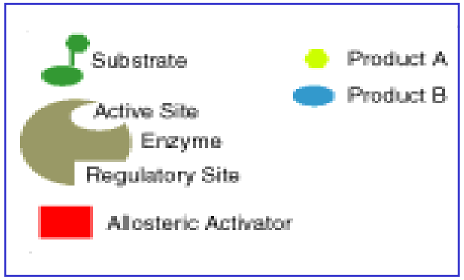
取得ＮＭＲ圖譜：
- 適當的實驗方法
- 標定ＮＭＲ譜線

分析圖譜結果：
- 鑑定化學(生化)分子
- 分子結構、動力學、反應機制……









Before using NMR

What's N, M, and R?

Properties of the Nucleus

Nuclear spin

Nuclear magnetic moments

The Nucleus in a Magnetic Field

Precession and the Larmor frequency

Nuclear Zeeman effect & Boltzmann distribution

When the Nucleus Meet the right Magnet

Nuclear Magnetic Resonance

◆ Properties of the Nucleus

Nuclear spin

• Nuclear spin is the total nuclear angular momentum quantum number. This is characterized by a quantum number I, which may be integral, half-integral or 0.

• Only nuclei with spin number $I \neq 0$ can absorb/emit electromagnetic radiation. The magnetic quantum number m_I has values of $-I$, $-I+1$, ...$+I$.
(e.g. for $I=3/2$, $m_I=-3/2, -1/2, 1/2, 3/2$)

1. A nucleus with an even mass A and even charge Z → nuclear spin I is zero

Example: ^{12}C, ^{16}O, ^{32}S → No NMR signal

2. A nucleus with an even mass A and odd charge Z → integer value I

Example: ^{2}H, ^{10}B, ^{14}N → NMR detectable

3. A nucleus with odd mass A → $I=n/2$, where n is an odd integer

Example: ^{1}H, ^{13}C, ^{15}N, ^{31}P → NMR detectable

Nuclear magnetic moments

Magnetic moment μ is another important parameter for a nuclei

$$\mu = \gamma \; I \; (h/2\pi)$$

I: spin number

h: Plank constant 6.626×10^{-34} joul-sec

γ: gyromagnetic ratio (property of a nuclei)

1H: I=1/2 , γ = 267.512×10^{6} rad $T^{-1}sec^{-1}$

13C: I=1/2 , γ = 67.264×10^{6}

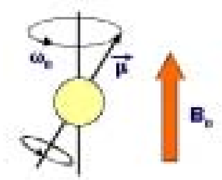
15N: I=1/2 , γ = 27.107×10^{6}

Precession and the Larmor frequency

- The magnetic moment of a spinning nucleus processes with a characteristic angular frequency called the Larmor frequency ω, which is a function of r and B_0

Larmor frequency $\omega = rB_0$

Linear precession frequency $\upsilon = \omega/2\pi = rB_0/2\pi$



Example: At what field strength do ^{1}H process at a frequency of 600.13MHz? What would be the process frequency for ^{13}C at the same field?

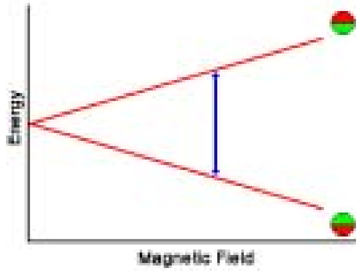
◆ The Nucleus in a Magnetic Field B_0

B_0 (the magnet of machine)

(1) Provide energy for the nuclei to spin

$E_i = -m_i B_0 (\gamma h/2\pi)$

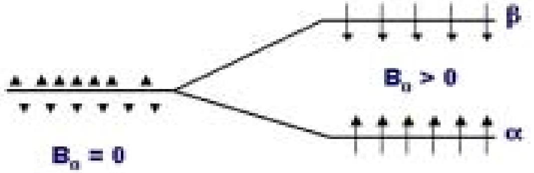
Larmor precession $\upsilon = \omega/2\pi = \gamma B_0/2\pi$



(2) Induce energy level separation (Zeeman effect & Boltzmann distribution)

The stronger the magnetic field B_0, the greater separation

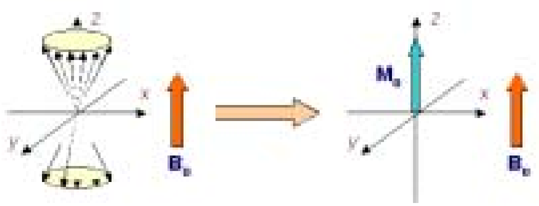
$P_{m=-1/2} / P_{m=+1/2} = e^{-\Delta E/kT}$



(3) The nuclei in both spin states are randomly oriented around the z axis.

$M_z = M_0$, $M_{xy} = 0$

(where M_0 is the net nuclear magnetization)

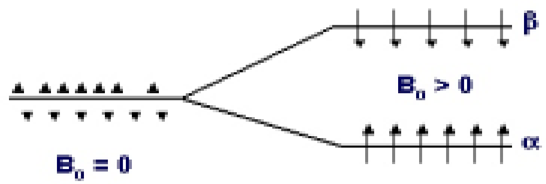


◆ When the Nucleus Meet the "right" Magnet: N. M. Resonance

B*$_1$*(the irradiation magnet, current induced)

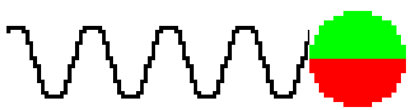
(1) Induce energy for nuclei to absorb, but still spin at ω or $\upsilon_{precession}$

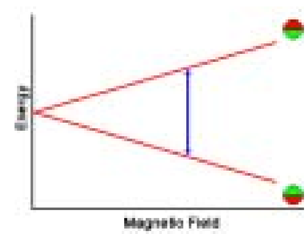
$$E_{induced}=\Delta E=\gamma h B_0/2\pi=h\upsilon_{precession}$$



$\Delta E = E_{induced}$

And now, the spin jump to the higher energy (from m=1/2→m= – 1/2)





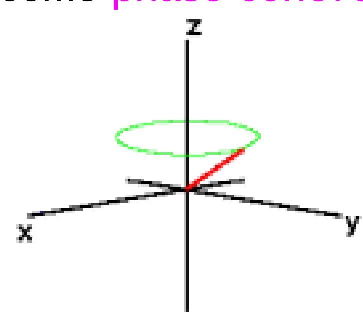
m= –1/2

m= 1/2

(2) All of the individual nuclear magnetic moments become phase coherent, and the net M process around the z axis at a angel α

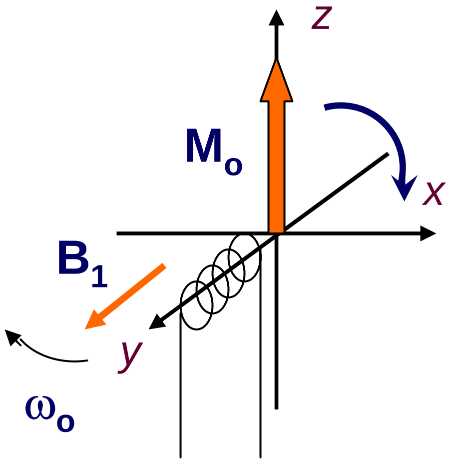
$M_z=M\cos\alpha$

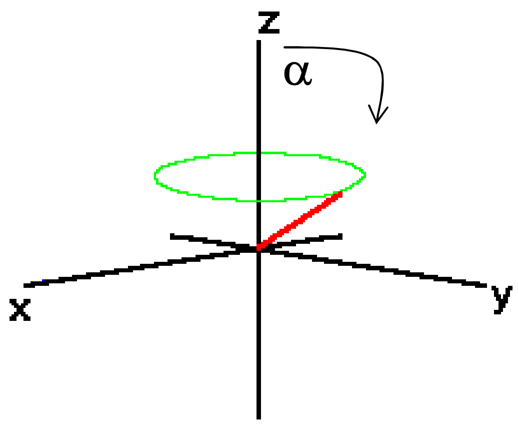
$M_{xy}=M\sin\alpha$.



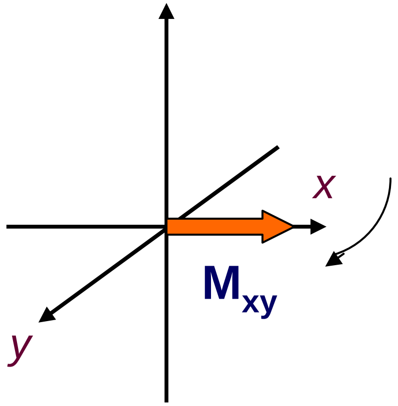
What happen during irradiation

When irradiation begins, all of the individual nuclear magnetic moments become phase coherent, and this phase coherence forces the net magnetization vector M_0 to process around the z axis. As such, M has a component in the x, y plan, $M_{xy}=M\sin\alpha$. α is the tip angle which is determined by the power and duration of the electromagnetic irradiation.





α deg pulse



90 deg pulse

Hint: that's why we need to calibrate 90º pulse !!

What happen after irradiation ceases

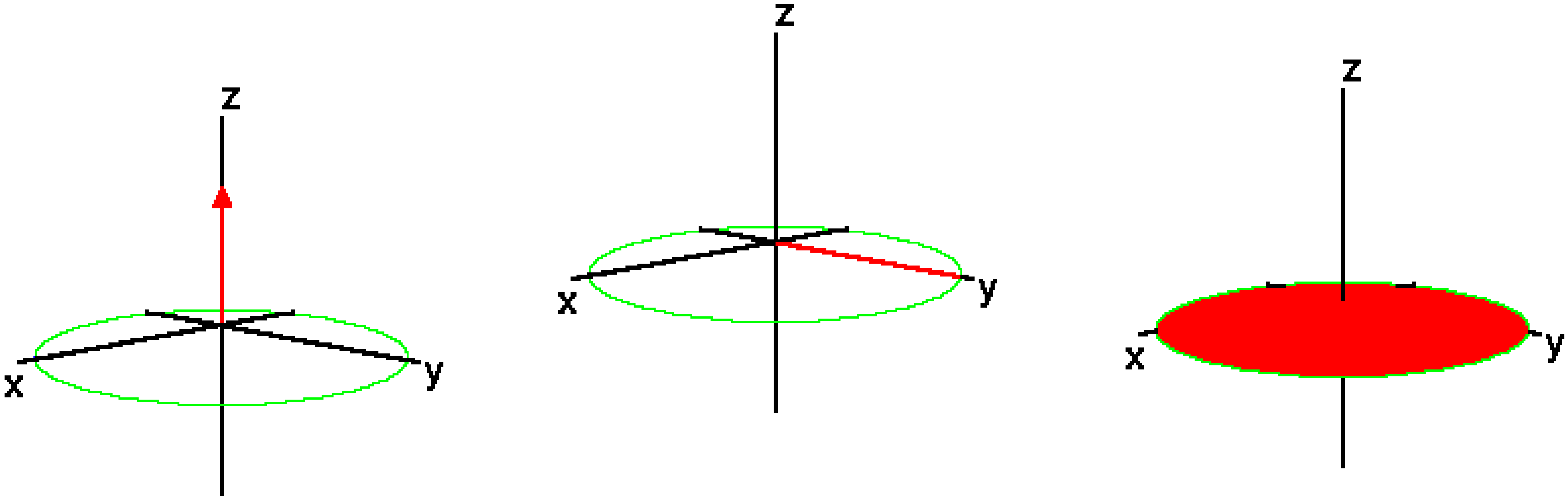
•After irradiation ceases, not only do the population of the states revert to a Boltzmann distribution, but also the individual nuclear magnetic moments begin to lose their phase coherence and return to a random arrangement around the z axis.

(NMR 的光譜其實就是在紀錄這個過程!!)

•This process is called “relaxation process” (弛緩現象)

•There are two types of relaxation process :

- T1(spin-lattice relaxation)
- T2(spin-spin relaxation)



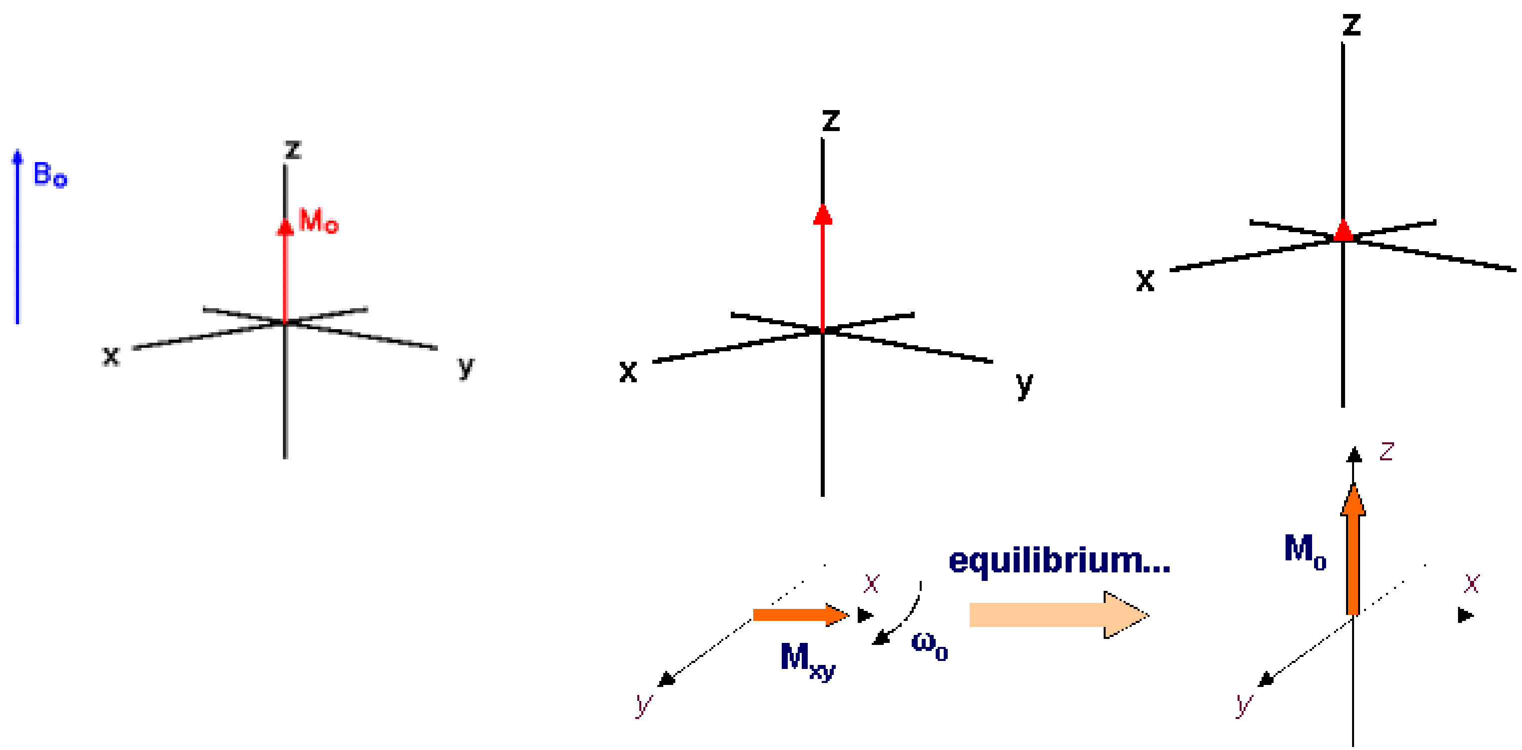
T1 (the spin lattice relaxation)

- How long after immersion in a external field does it take for a collection of nuclei to reach Boltzmann distribution is controlled by T1, the spin lattice relaxation time.

(考慮波茲曼分布的效應為主)

- Lost of energy in system to surrounding (lattice) as heat (能量釋放的過程)
- It's a time dependence exponential decay process of Mz components

$$dM_z/dt=-(M_z-M_{z,eq})/T1$$



T2 (the spin –spin relaxation)

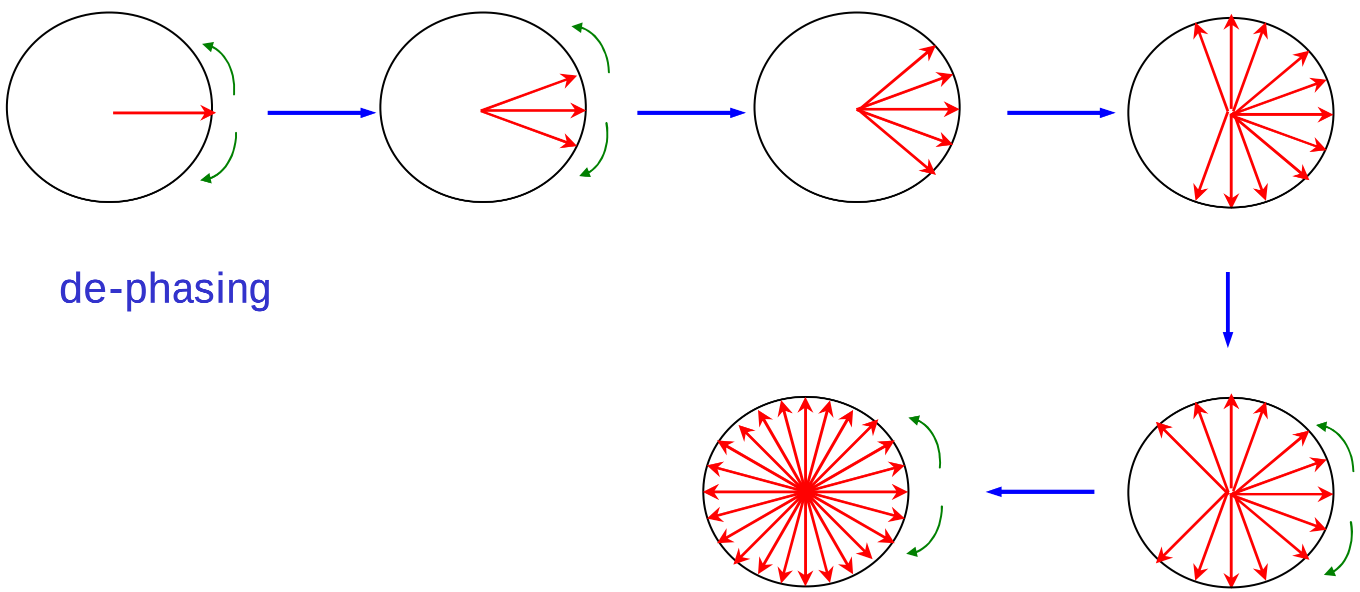
- This process for nuclei begin to lose their phase coherence and return to a random arrangement around the z axis is called spin-spin relaxation.

(考慮自旋方位由同一方向又回到 random 的過程)

- The decay of M_{xy} is at a rate controlled by the spin-spin relaxation time T2.

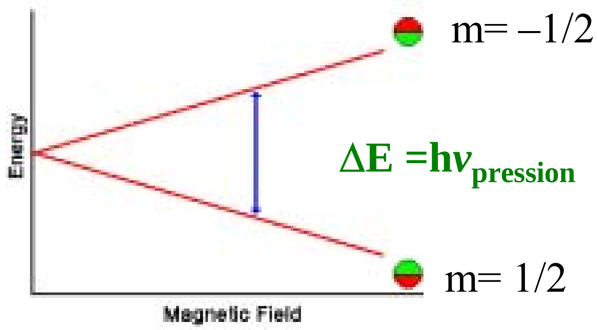
$dM_x/dt=-M_x/T2$

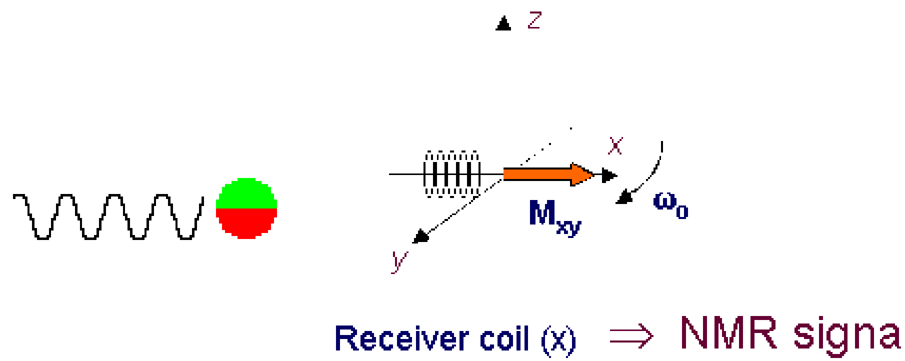
$dM_y/dt=-M_y/T2$



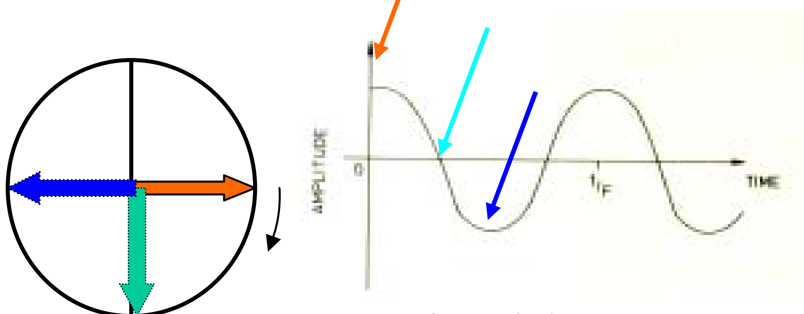
◆ Collecting NMR signals

•The detection of NMR signal is on the xy plane. The oscillation of Mxy generate a current in a coil , which is the NMR signal.

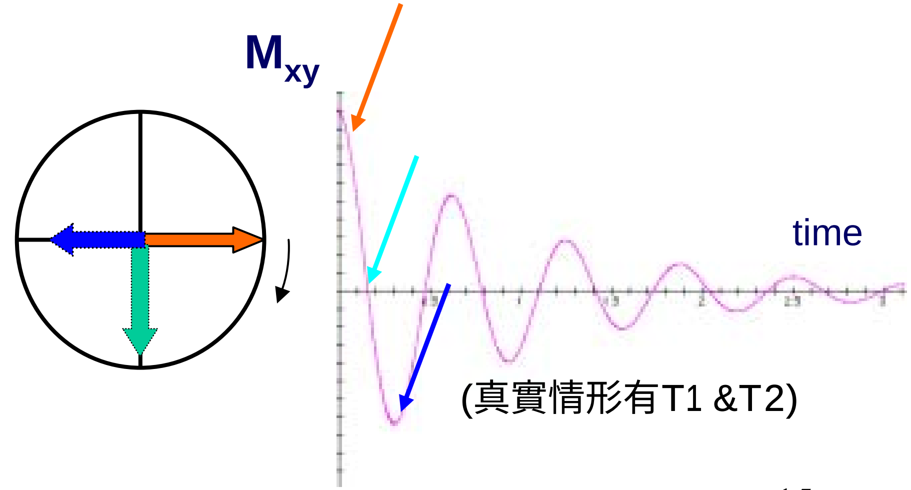




•Due to the "relaxation process", the time dependent spectrum of nuclei can be obtained. This time dependent spectrum is called "free induction decay" (FID)



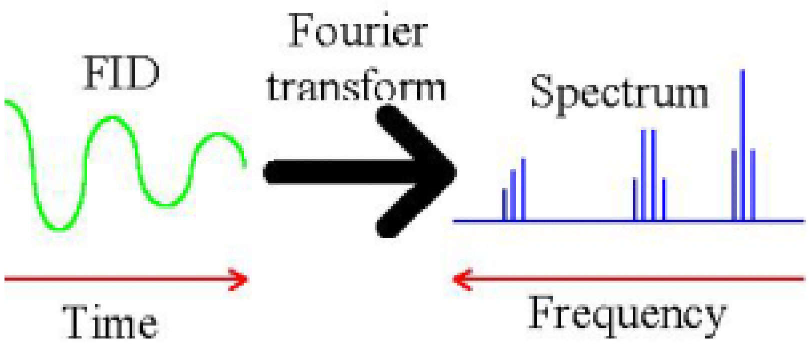
(如果沒有 relaxation)

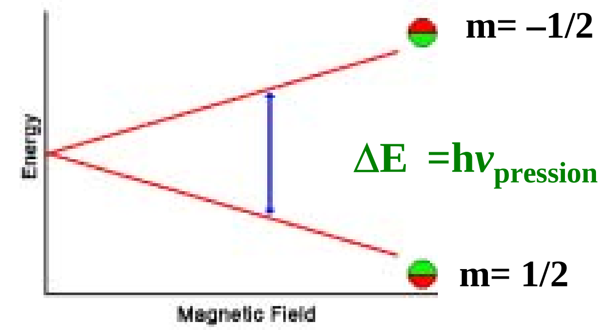


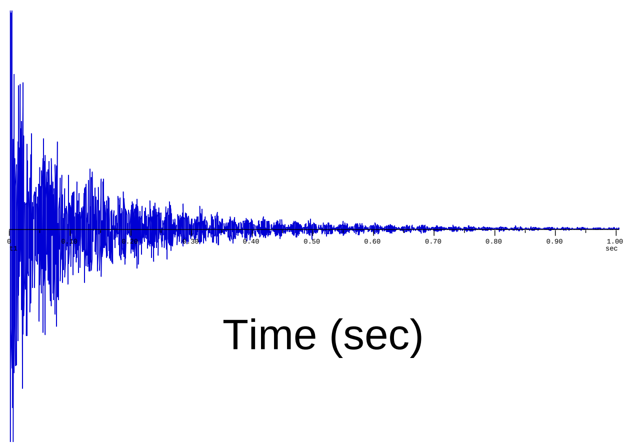
(真實情形有T1 &T2)

NMR Data Processing

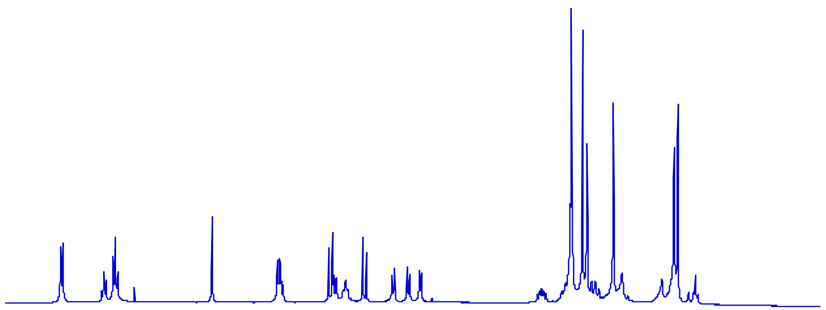
● The FID (free induction decay) is then Fourier transform to frequency domain to obtain $\upsilon_{pression}$ (chemical shift) for each different nuclei.







Time (sec)



Frequency (Hz)

Example of NMR signals

- It's easy to understand that different nucleus "type" will give different NMR signal. ($\nu = w/2\pi = \gamma B0/2\pi$, γ: gyromagnetic ratio is the property of a nuclei.)

- However, it is very important to know that for same "nucleus type", but "different nucleus" could generate different signal. This is also what make NMR useful and interesting.

- Electron surrounding each nucleus in a molecule serves to shield that nucleus from the applied magnetic field. This shielding effect cause different ν in the spectrum

$B_{eff}=B_0-B_i$ where B_i induced by cloud electron

$B_i = \sigma B_0$ where σ is the shielding constant

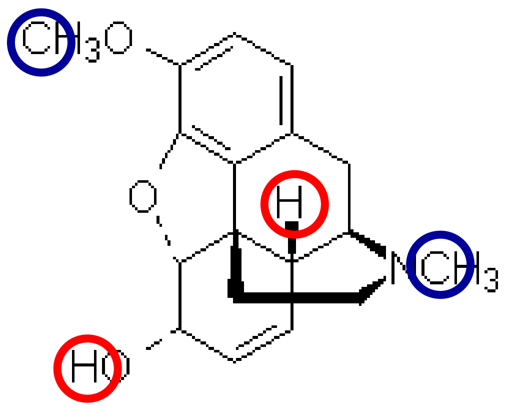
$B_{eff}=(1-\sigma)\ B_0$

$\nu_{precession} = (rB_0/2\pi)\ (1-\sigma)$

$\sigma = 0$ → naked nuclei

$\sigma > 0$ → nuclei is shielded by electron cloud

$\sigma < 0$ → electron around this nuclei is withdraw , i.e. deshielded



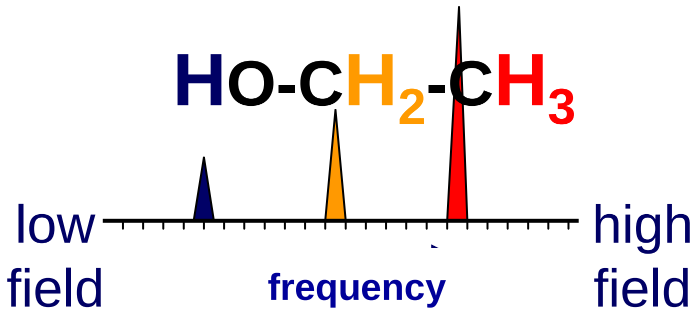
Basic of Assignment

$\nu_{precession} = (rB_0/2\pi)(1-\sigma) = \nu_{0,\ precession}(1-\sigma)$

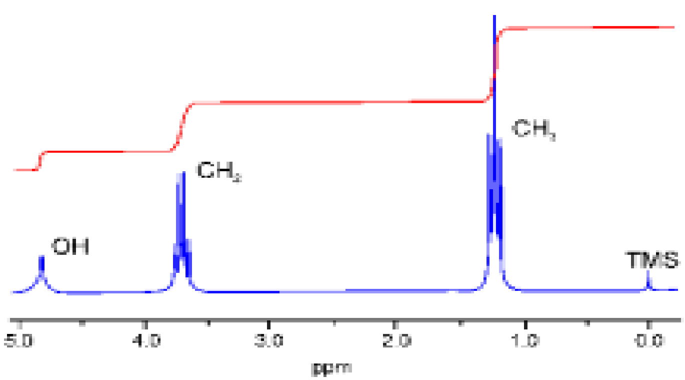
$\sigma = 0$ → naked nuclei

$\sigma > 0$ → nuclei is shielded by electron cloud

$\sigma < 0$ → electron around this nuclei is withdraw , i.e. deshielded



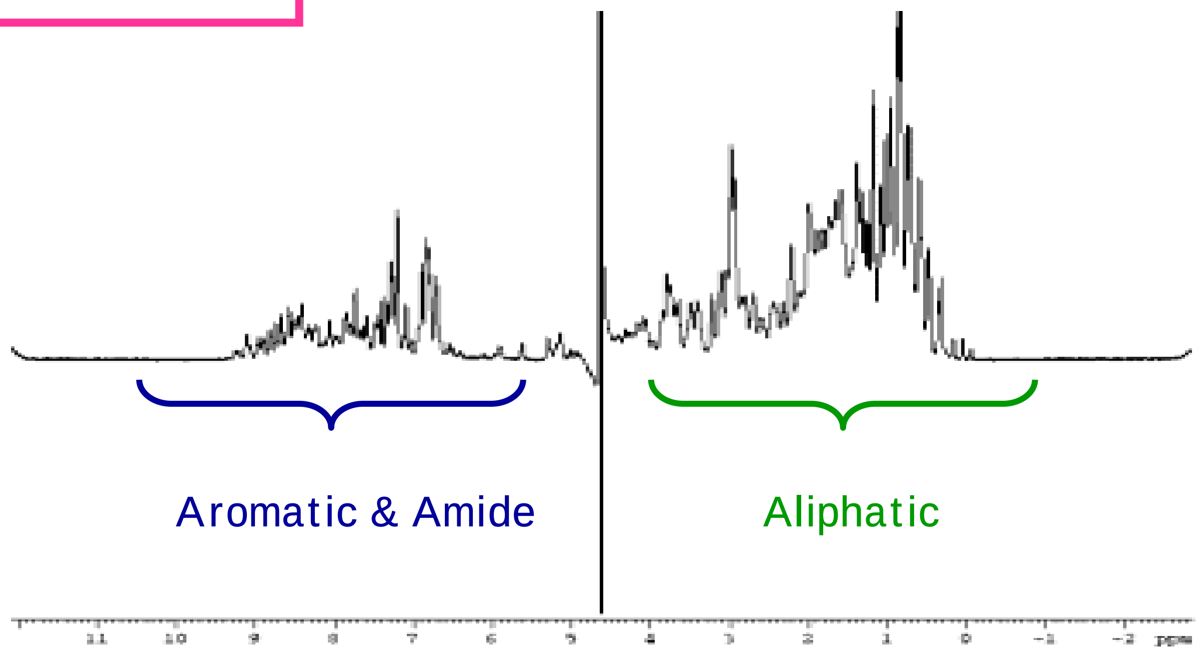
$ppm \cdot 10^{-6} = \Delta\nu/\nu_0 = -\sigma$

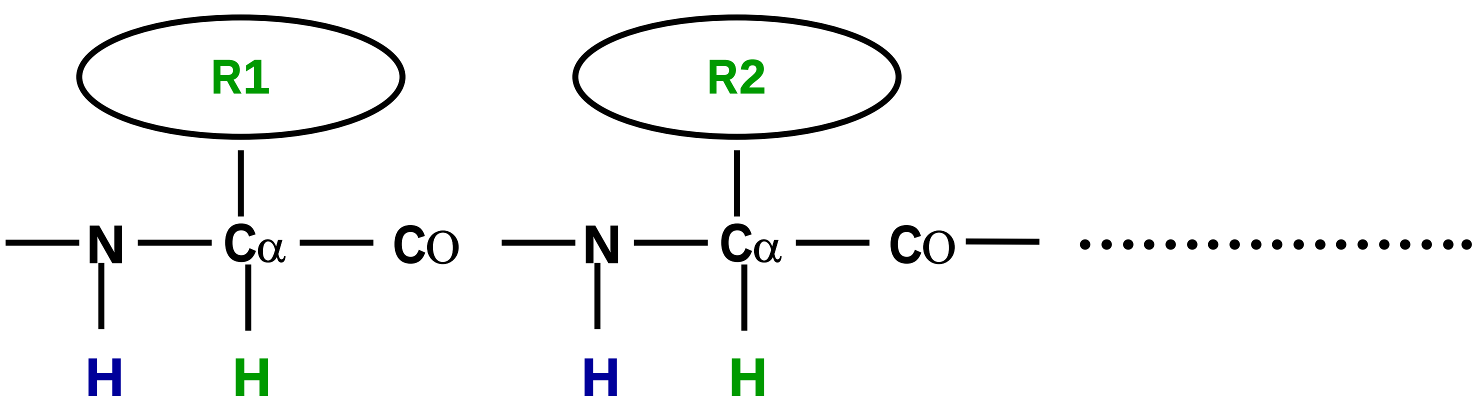


Introduction of NMR Experiments

Homo Nuclear 1D NMR

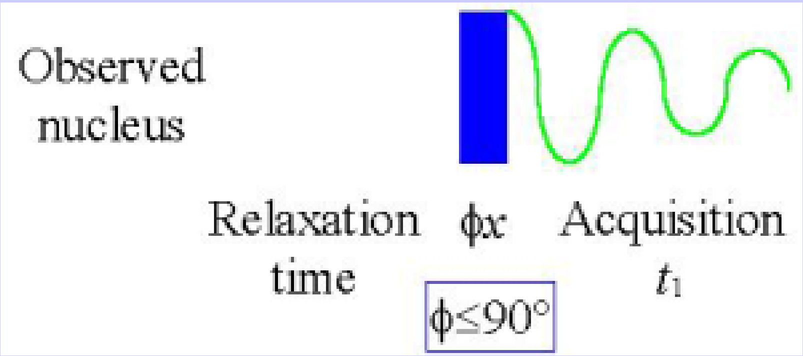
1D one pulse 1H

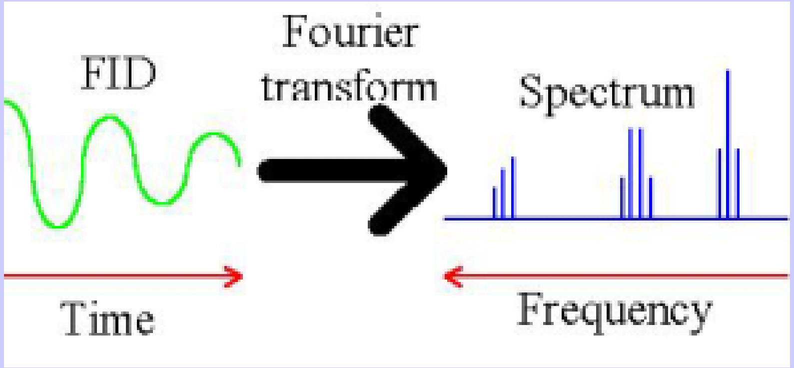




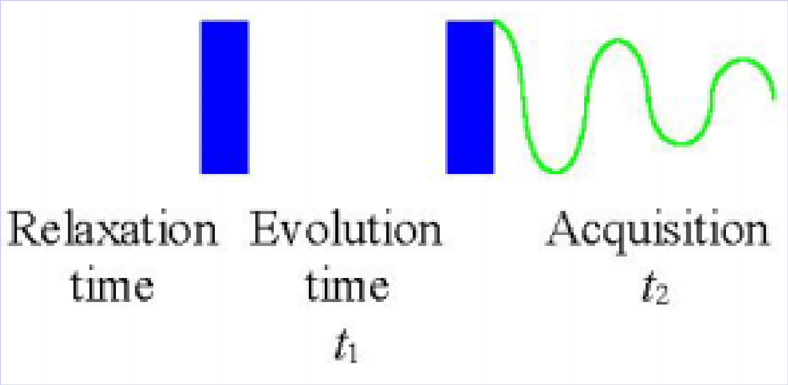
Homo/Hetro Nuclear 2D NMR

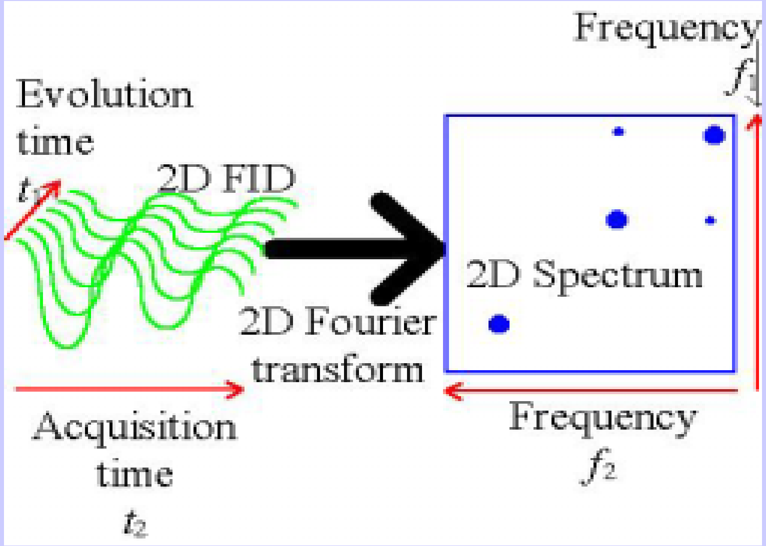
Basic 1D Experiment





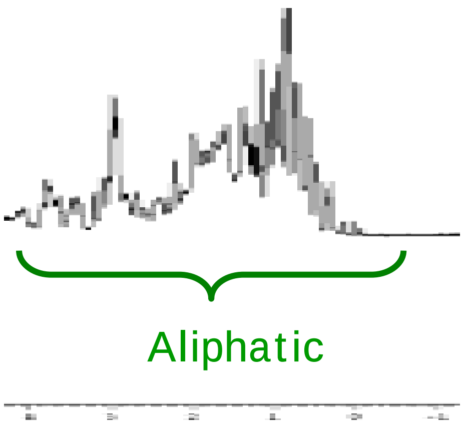
Basic 2D Experiment

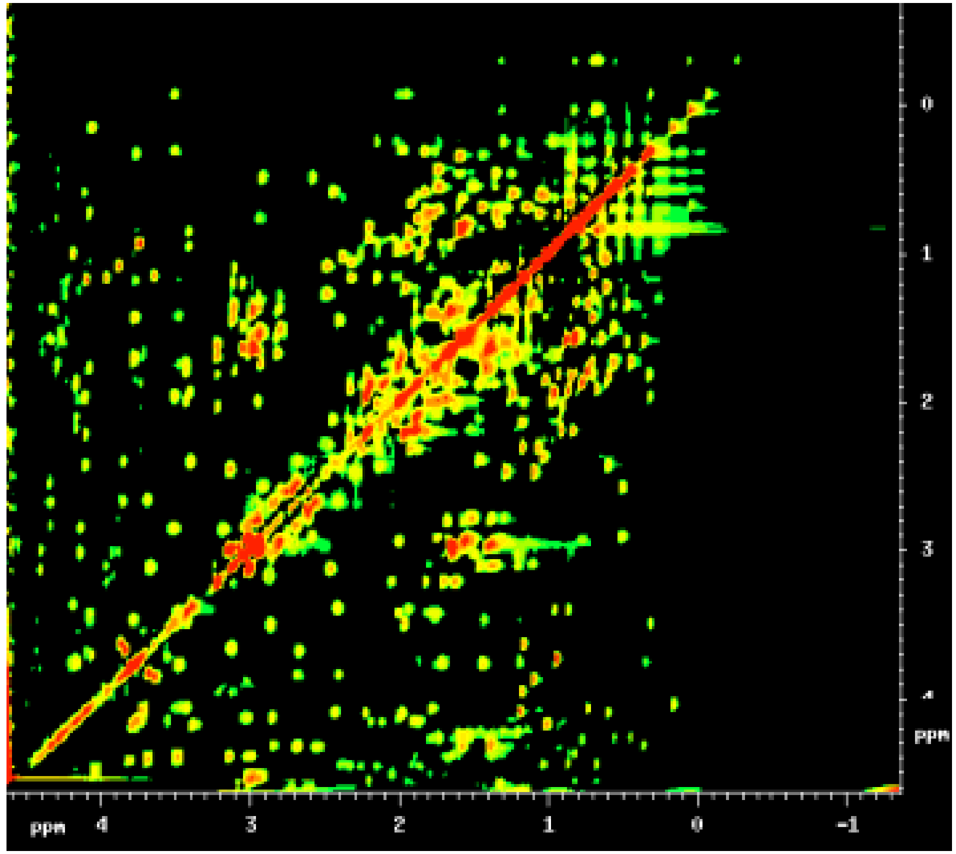


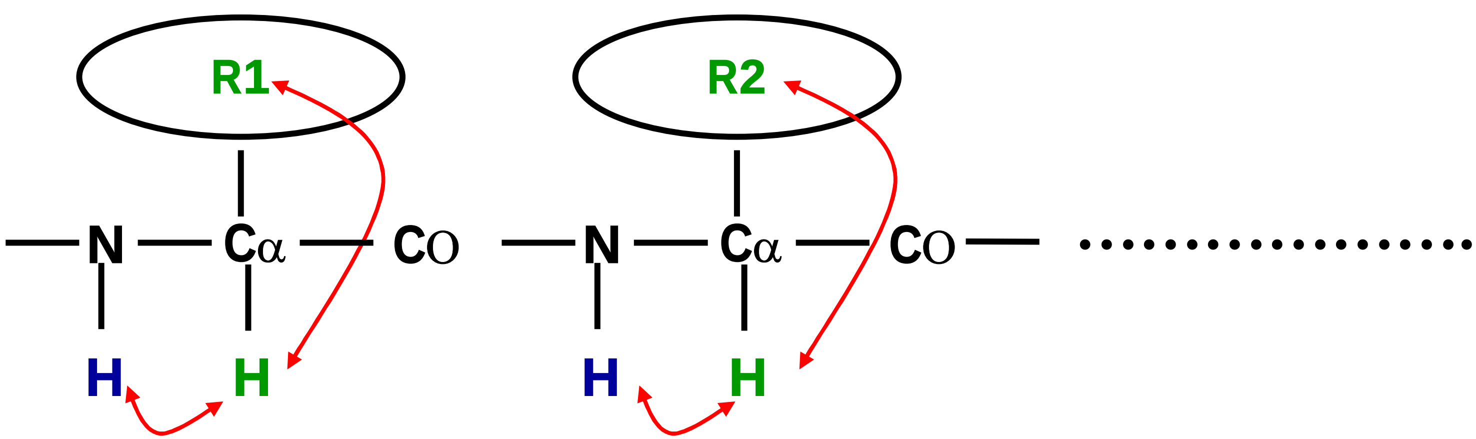


2D Homo Nuclear

1H - 1H



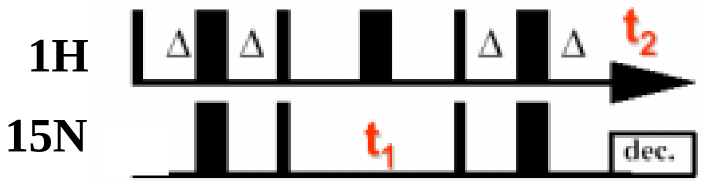


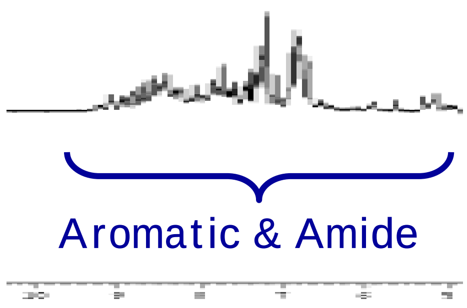


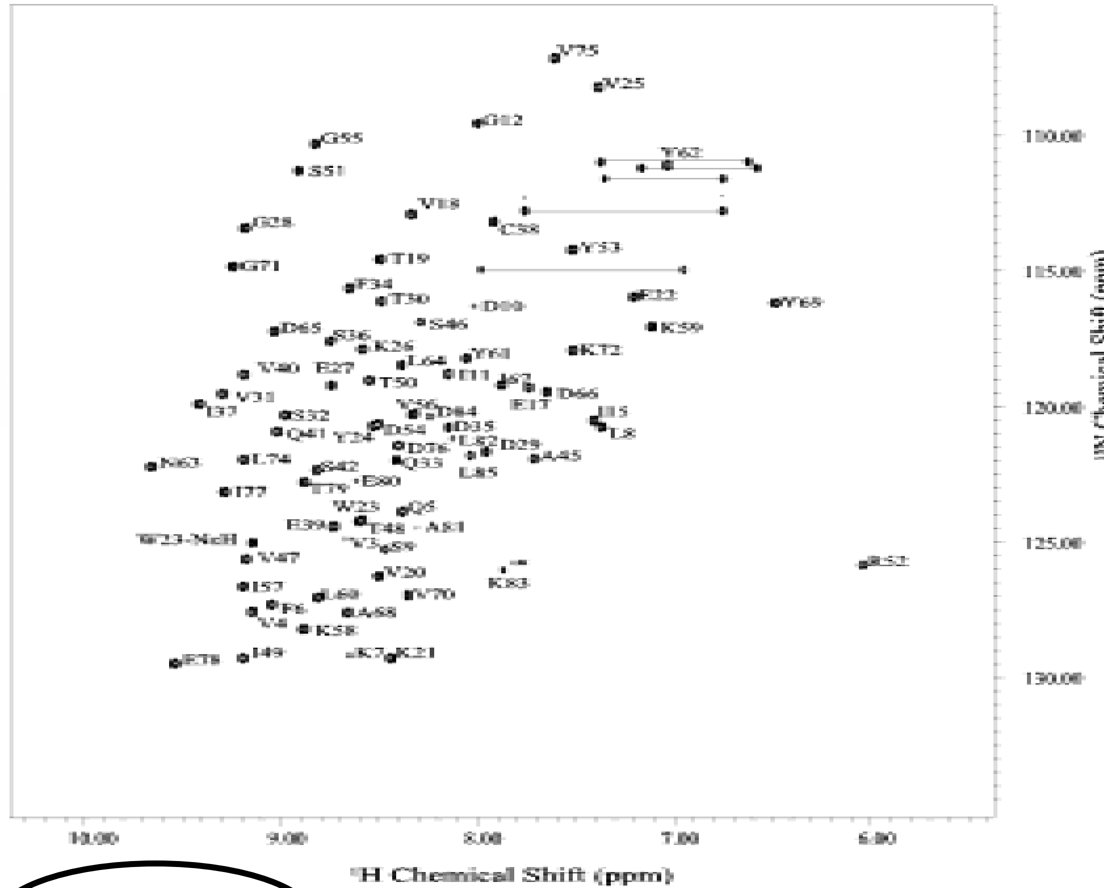
2D Hetero Nuclear

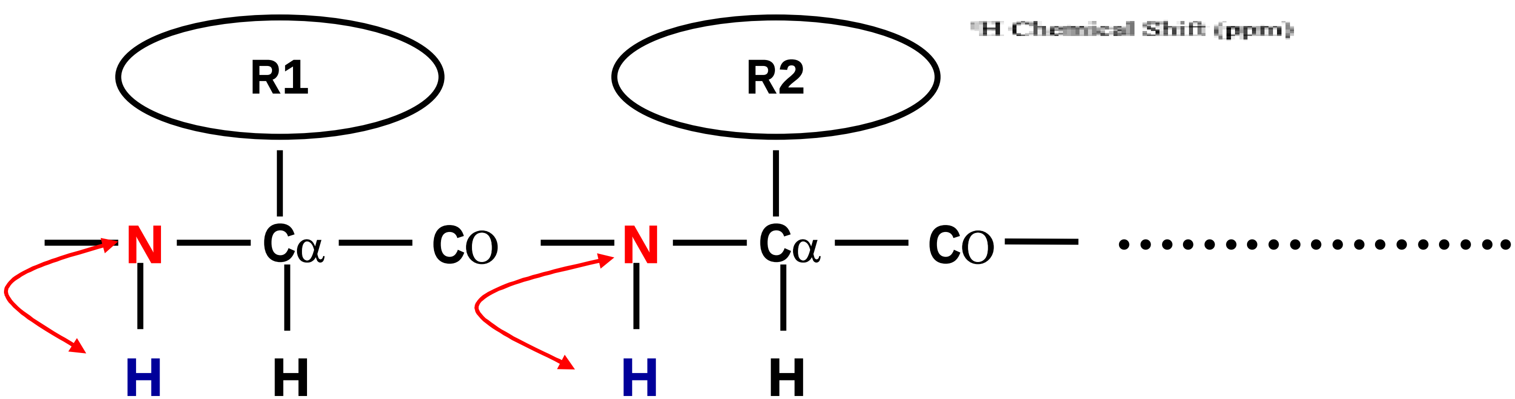
1H-15N

2D HSQC



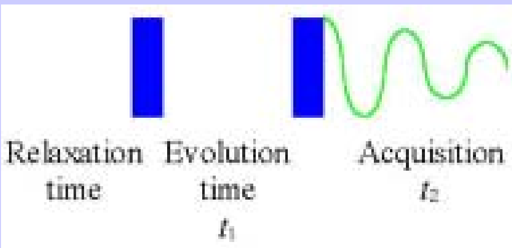






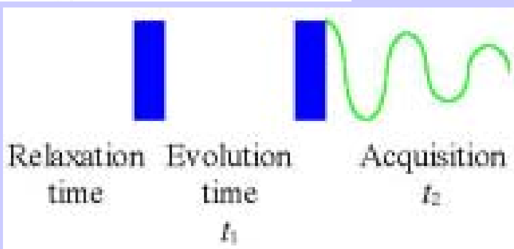
Multi-Dimensional NMR

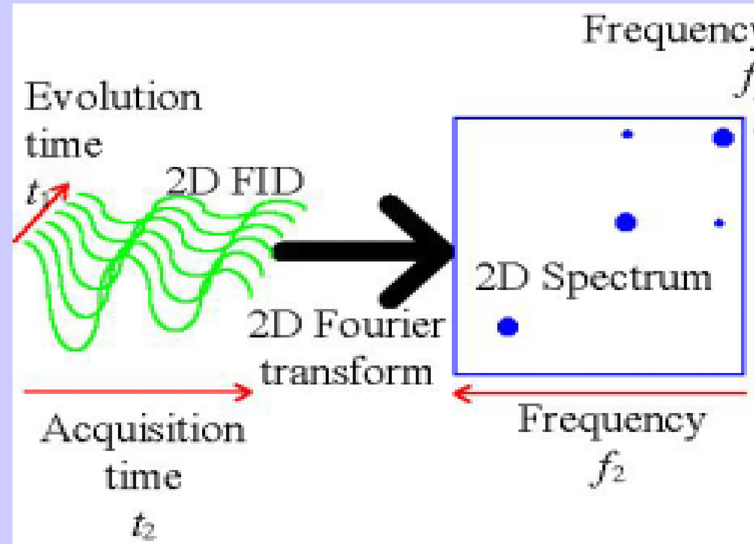
Basic 3D Experiment



2D

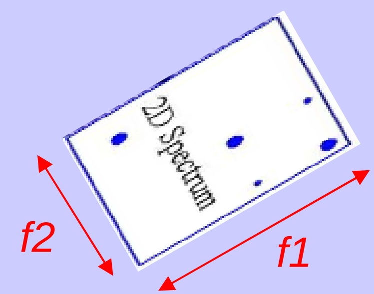
2D

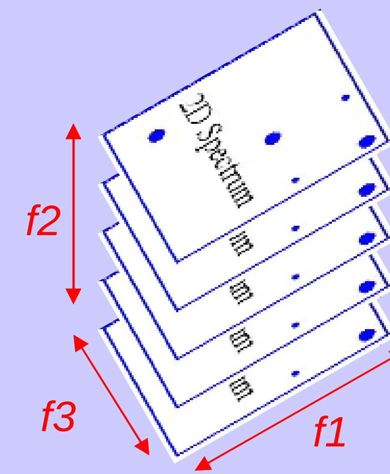




3D

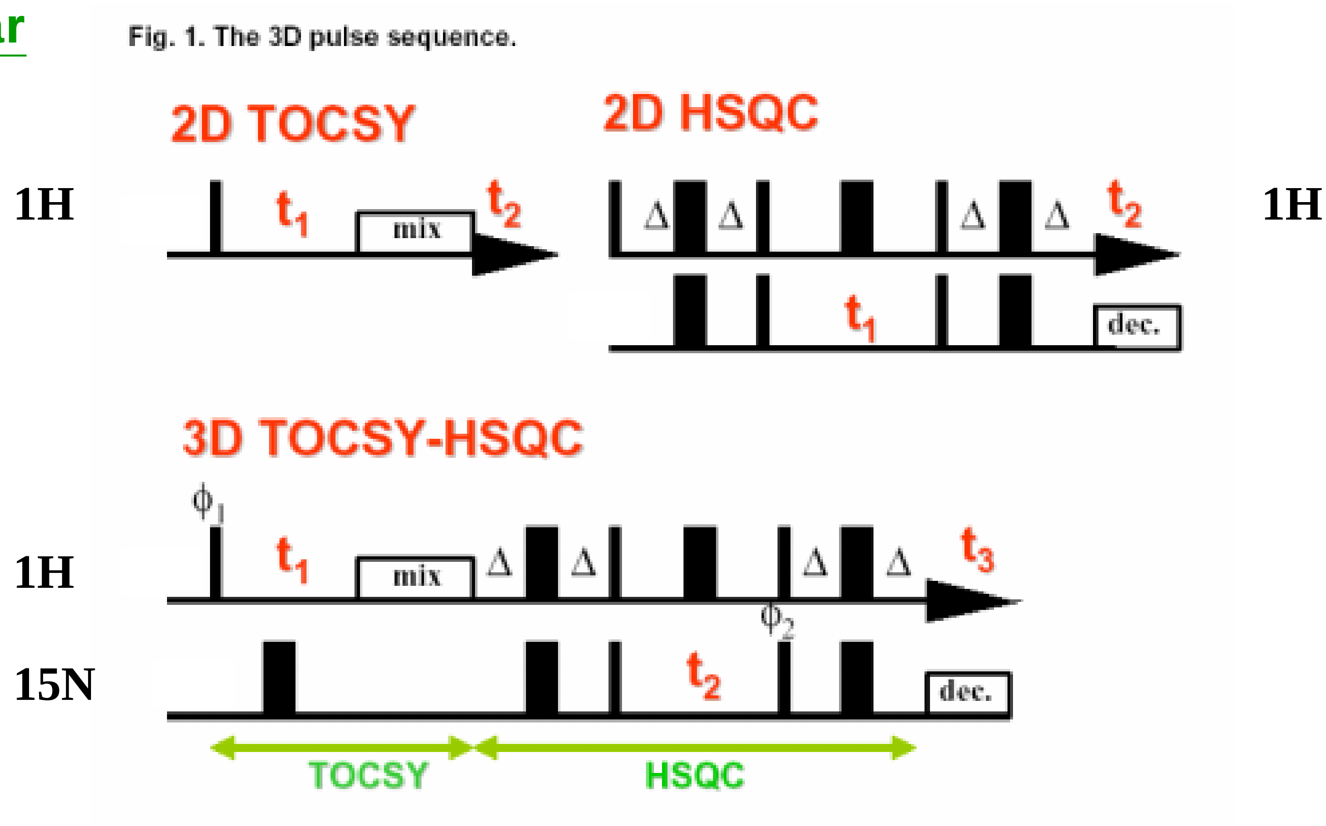
t1 t2 t3

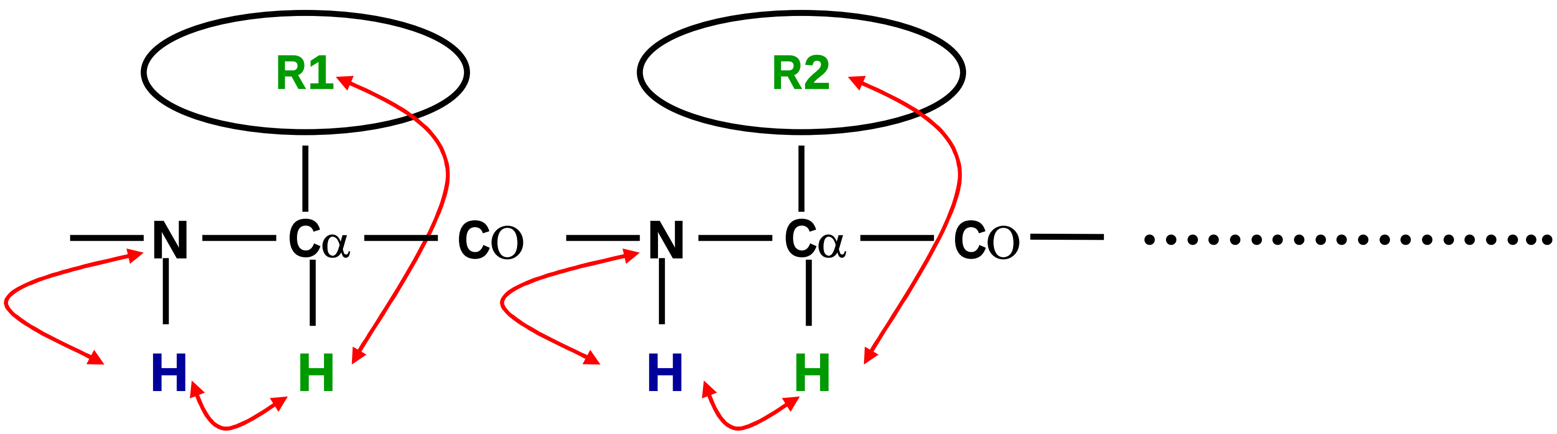




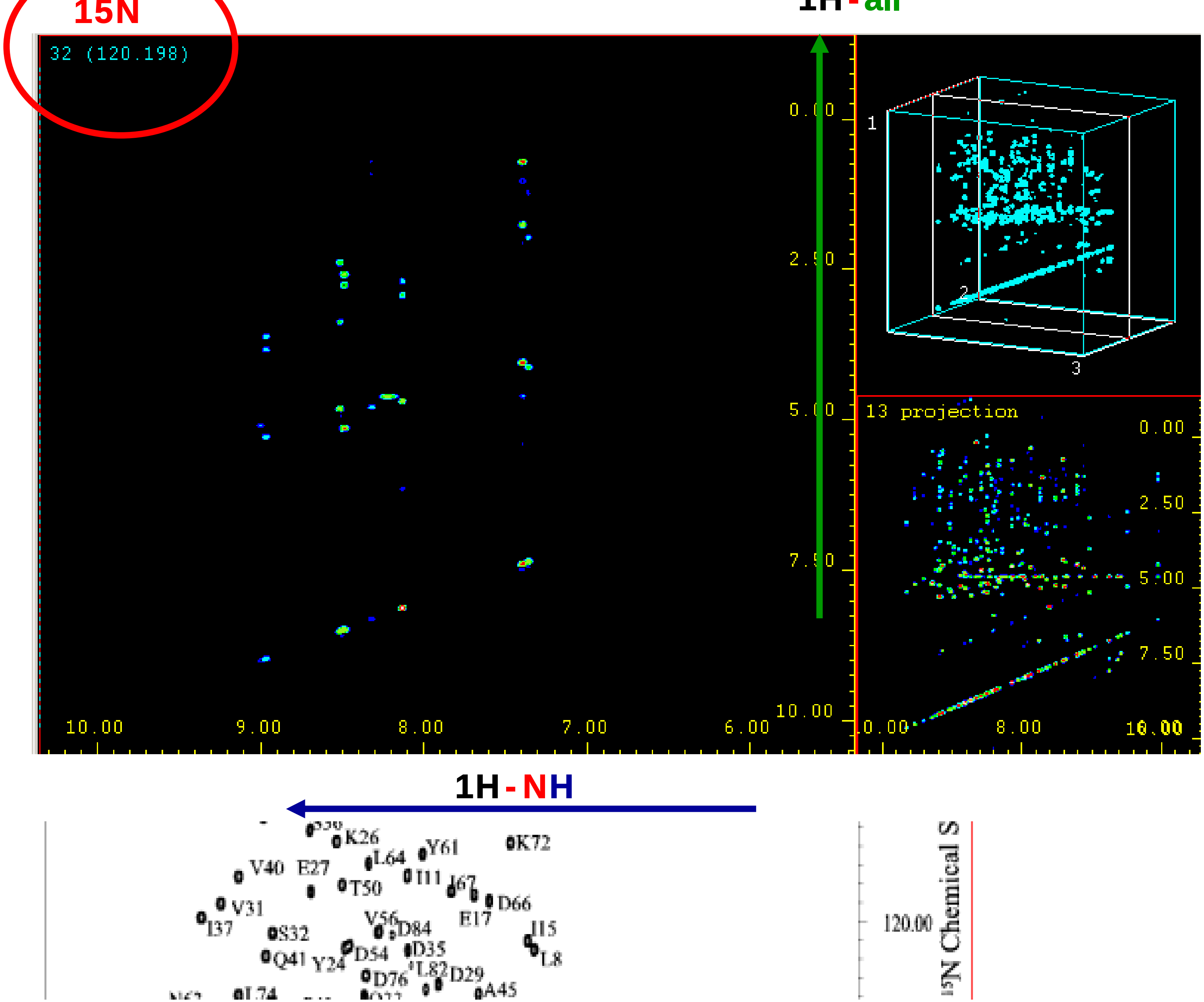
3D Hetero Nuclear

1H - 15N - 1H

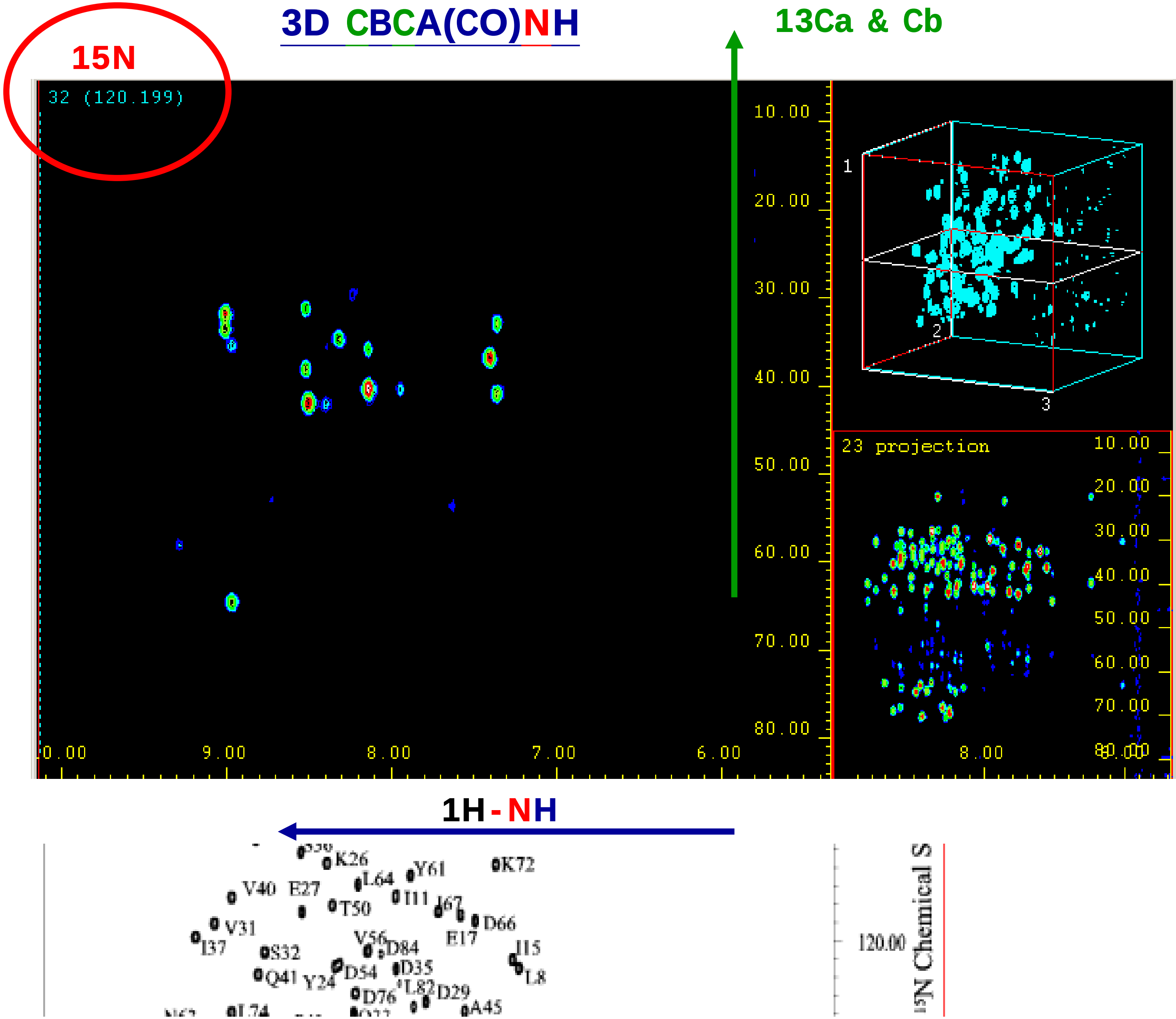




3D 15N-edit TOCSY-HSQC



3D CBCA(CO)NH


15N
32 (120.199)
13Ca & Cb
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
9.00
8.00
7.00
6.00
1
2
3
23 projection
1H - NH
K26
Y61
K72
L64
V40
E27
T50
I11
D66
V31
I37
S32
E17
D84
I15
D35
Q41
Y24
D54
L8
D76
L82
D29
A45
120.00
15N Chemical S

Set up 1D/homo Nuclear 2D

(Bruker AV system)

Before go into NMR LAB

Overview of NMR Facility

AV500 in IBMS : 5mm TXI-Z ($^{1}H/^{15}N/^{13}C$, with Z gradient) only

5mm TXI-Z CryoProbe (not available yet)

AV600 in IBMS: 5mm QXI-Z ($^{1}H/^{15}N/^{13}C$ /^{31}P)

5mm TXI-Z CryoProbe (not available yet)

AV600 in CHEM: 5mm BBO & TXI-Z ($^{1}H/^{15}N/^{13}C$, with Z gradient)

DRX600 in IBMS: 5mm TXI-XYZ ($^{1}H/^{15}N/^{13}C$, with XYZ gradient) and others

5mm : ^{1}H , $^{1}H/^{19}F$, BBO, TXI($^{1}H/^{15}N/^{13}C$) , TXI-Z ($^{1}H/^{3}C/^{31}P$)

8mm : TXI ($^{1}H/^{13}C/^{15}N$) 8mm with Z gradient

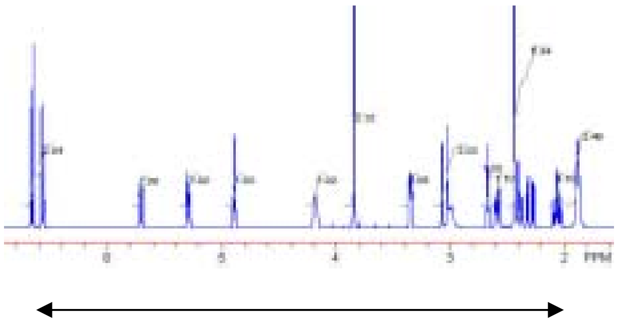
10mm: ^{1}H , ^{1}H /^{19}F , BBO

AV800 in IBMS (not available yet) : 5mm TXI-Z & CryoProbe

Definition of some AQ Commands & parameters

edc,new	**edit current data set or generate a new data set**
eda.ased	**edit AQ parameters (eda: shows all, ased: shows required only)**
rga	**auto optimize rg value**
zg	**zero memory, and start to collect FID (go)**
go	**start to collect FID and add signals to the previous memory**
stop/halt	**stop the active job (currently AQ job)**
kill	**kill active job (can choose several jobs)**
o1.o2,o3	**center frequency of the spectrum for nuclear at f1 channel (ex: 1H), f2 channel (ex:13C), and f3 channel (ex:15N)**
sw	**spectrum width (1 sw : F1 dimension, 2 sw: F2 dimension…….)**
td	**number of points for FID collection(1 td: F1 dimension, 2 td: F2 dimension….)**
d1	**relaxation time (usually > 5* T1)**
ns	**number of scan**
ds	**dummy scan**
rg	**receiver gain (usually use the value calculated by rga)**

1D

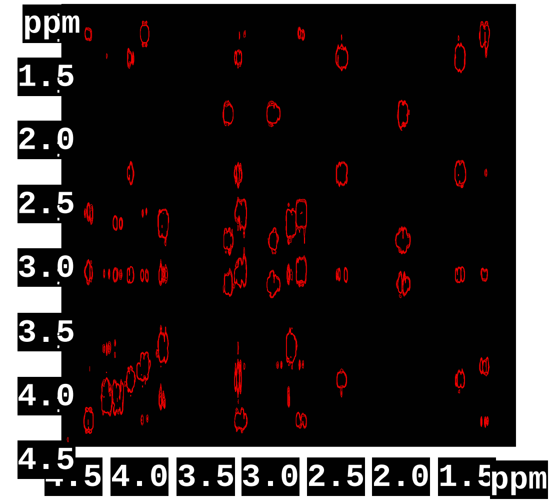


center (o1) at 4.5ppm

sw=6ppm

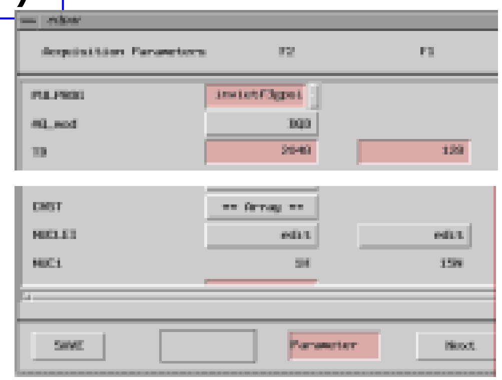
1.5ppm (=o1-sw/2) to 7.5ppm(=o1+sw/2)

2D/homo



F1 dimension !!!

F2 dimension !!!



F2: H (f1 channel)

O1(f1 channel):3ppm

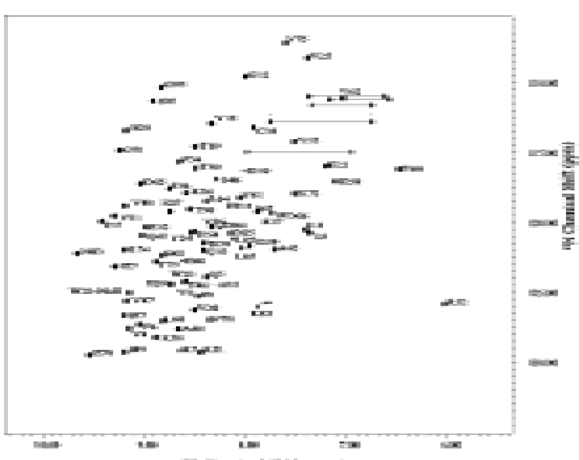
2 sw(F2 dimension): 5ppm

F1: H (f1 channel)

O1(f1 channel): 3ppm

1 sw(F1 diemnsion): 5ppm

2D/hetero



F2: H (f1 channel)

O1(f1 channel):8ppm

2 sw(F2 dimension): 5ppm

F1: N (f3 channel)

O3(f3 channel): 120ppm

1 sw(F1 diemnsion): 40ppm

Loading Sample

1. The best condition for sample? → . Probes, Temperature, Sample position

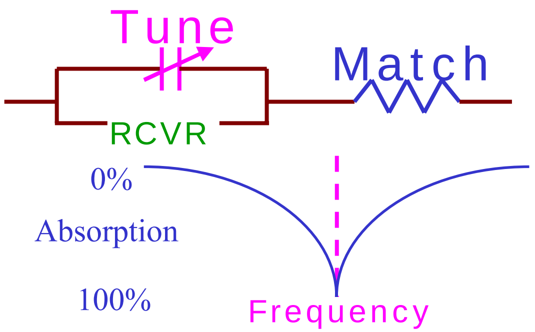
   Selecting the right probe : inner coil is observe coil and outer coil is decoupling

   Example: better 13C sensitivity should use BBO (13C: inner, 1H: outer), but if for better 1H, "inversed probe" (1H: inner, others: outer) is recommended

2. The best condition for NMR? → Wobble : Tune & Match

   Tuning is the process of adjusting freq. until it coincides with the desired frequency

   Matching is the process of adjusting the impedance of the resonant circuit



3. The best condition for field? → Lock and shim

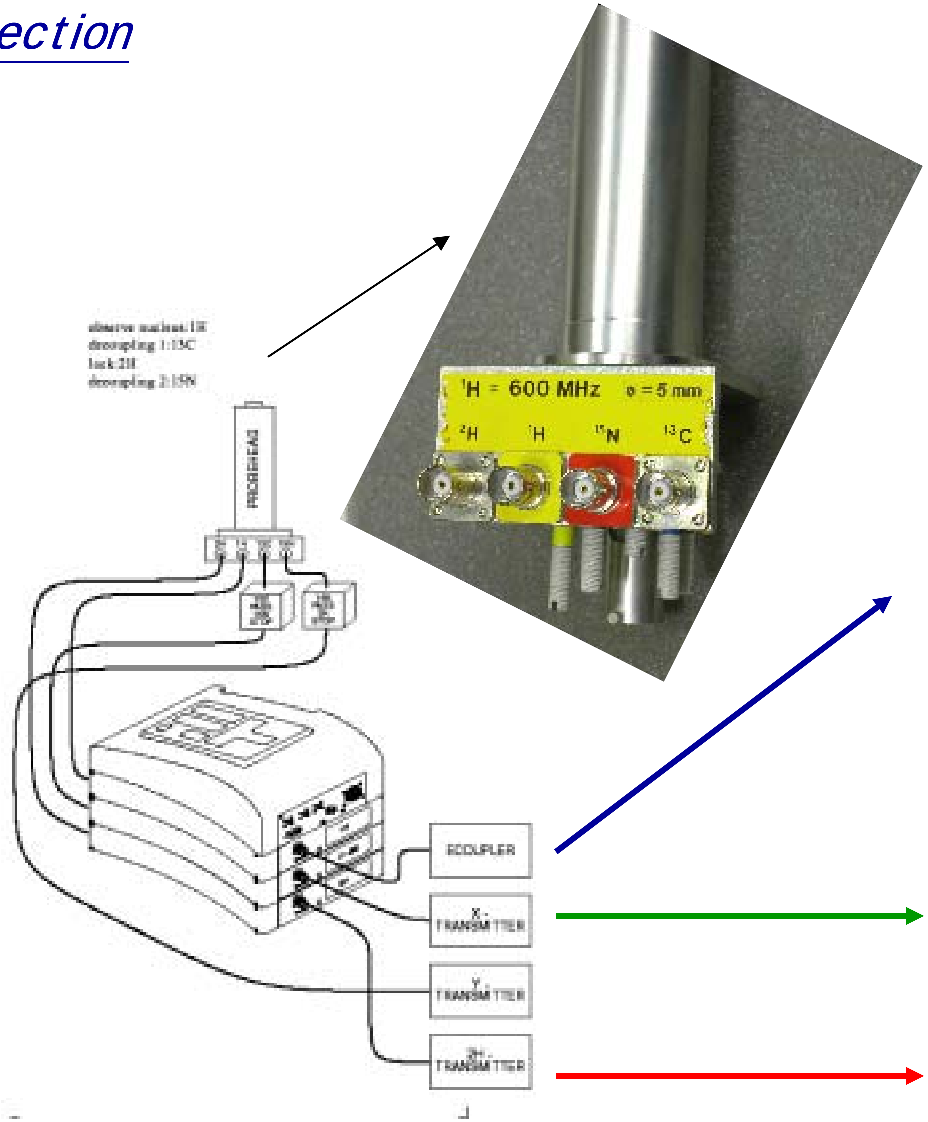
   Deuterium lock means the long term stability of the magnetic field is achieved

   The shims (coils) are small magnetic fields used to adjust the homogeneity of the field

4. Ready to go! (LAB work sheet)

In the NMR LAB

Hardware Connection



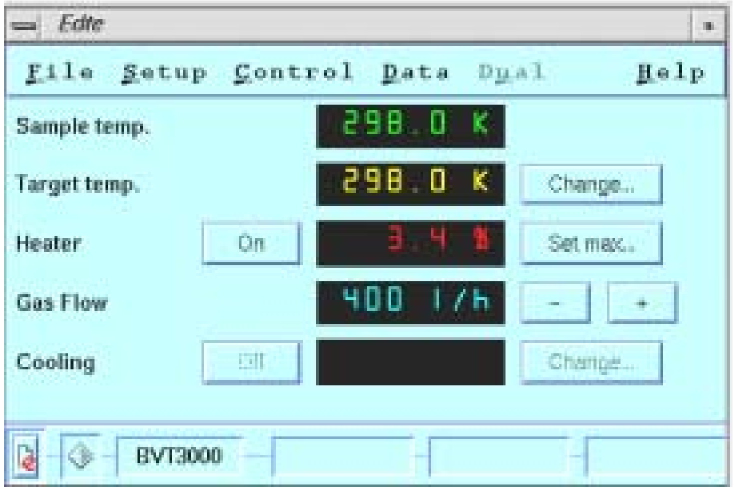
1H LNA

XBB19F

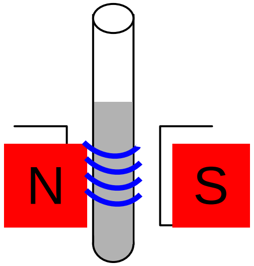
2H

Loading Sample

1. **Set up temperature : edte (edit temperature)**

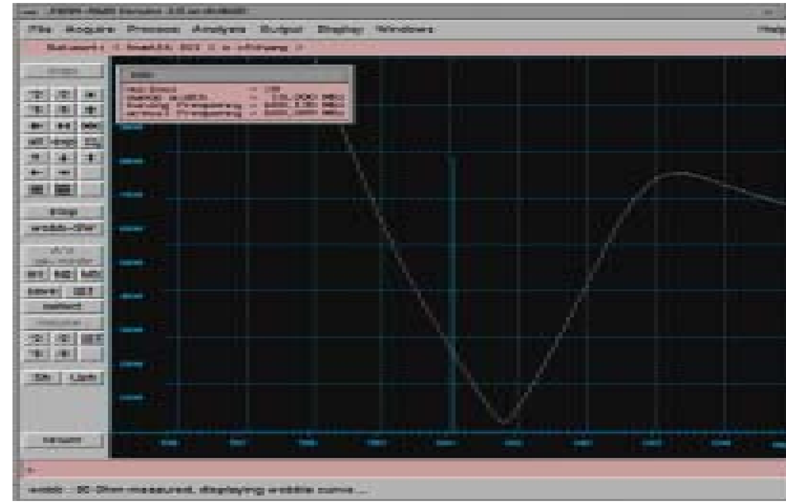


2. **Adjust sample position**



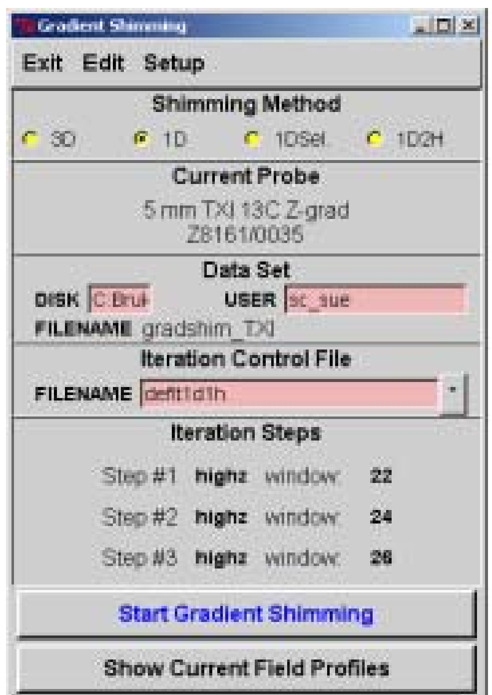
Hardware Adjustment

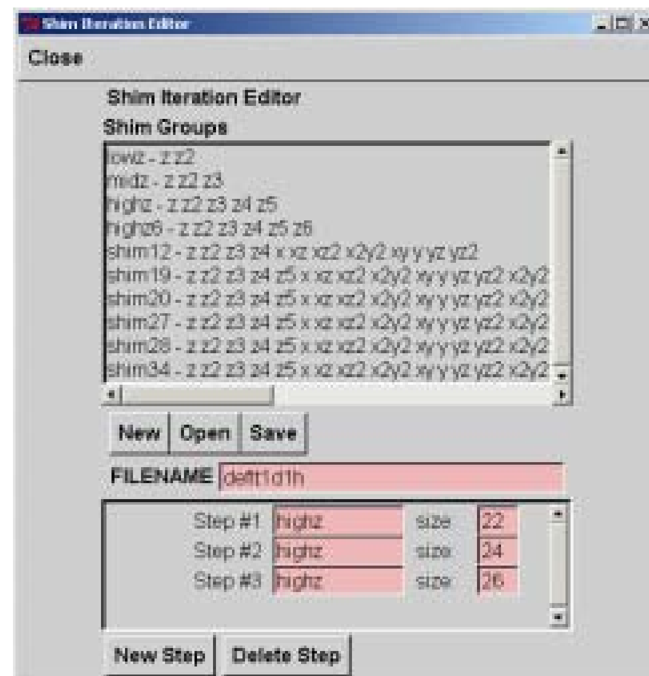
wobble



lock

gradshim : gradient shimming





Set up Experiment

Easy steps for beginner (1D):

1. Type “rpar” <enter> to load an appropriate parameter set
2. Type “ns” <enter> to input number of scan
3. Type “rga” <enter> to find appropriate receiver gain
4. Type “zg” <enter> to collect spectrum
5. Type “ft” <enter> to do Fourier Transfer
6. Click on phase to phase spectrum
7. Click on return, then save to save the spectrum
8. Print out the spectrum
9. Save your data on floppy

Define middle of the spectrum: O1

1. “rpar “ <std_1D_1H_ZG>
2. “zg” → “ft” → <phase>
3. Click on <utility> to define O1 position at middle of the spectrum

Steps for optimize 90 deg pulse:

1. “rpar” <std_1D_1H_ZGPR> , and set O1 to H2O position (if not in H2O, just use std_1D_1H_ZG to determine 90 deg pulse)
2. Set pL1= 0db (or 10db or ? db), p1=5u
3. “rga” → “zg”→ “ft” →<phase> → <save>
4. Keep pL1, increase p1= expected 180 deg

   (or, keep p1=180 deg pulse length, decrease pL1=expected power level for p1 value)
5. “zg”→ “fp” →check if the spectrum almost become null, if not, repeat step 4
6. Now, you should have a table with 1H pulse information of your sample

• How to optimize condition? → For users: Follow Experiment Guide

- **Experiment Name: 2D 1H TOCSY MLEV17**
- **Experiment Type: H2O supression using 3-9-19 & gradient**
- **Standard Parameter Set: std_2D_TOCSY-MLEV**
- **Pulse Program: mlevgpph19**
- **AQ parameters to check**

1H pulses

pl1 (high power, ex: 0dB), p1 (90o pulse at pl1)

pl10 (low power), p6 (90o pulse at pl10 for mixing pulse, ex: 25u)

pl18 (low power for 3-9-19, ex: 10dB), p0,p27 (90o pulse at pl18)

p28 (trim pulse, ex:1m)

Others

d9 (mixing time,ex: 60-70ms)

d19 (=1/2d, d=distance of next null in Hz)

o1 (on H2O)

1 sw, 1td (for F1 dimension, H)

2 sw, 2 td (for F2 dimension, H)

d1, ns(=2*n), ds(>=16)

rg

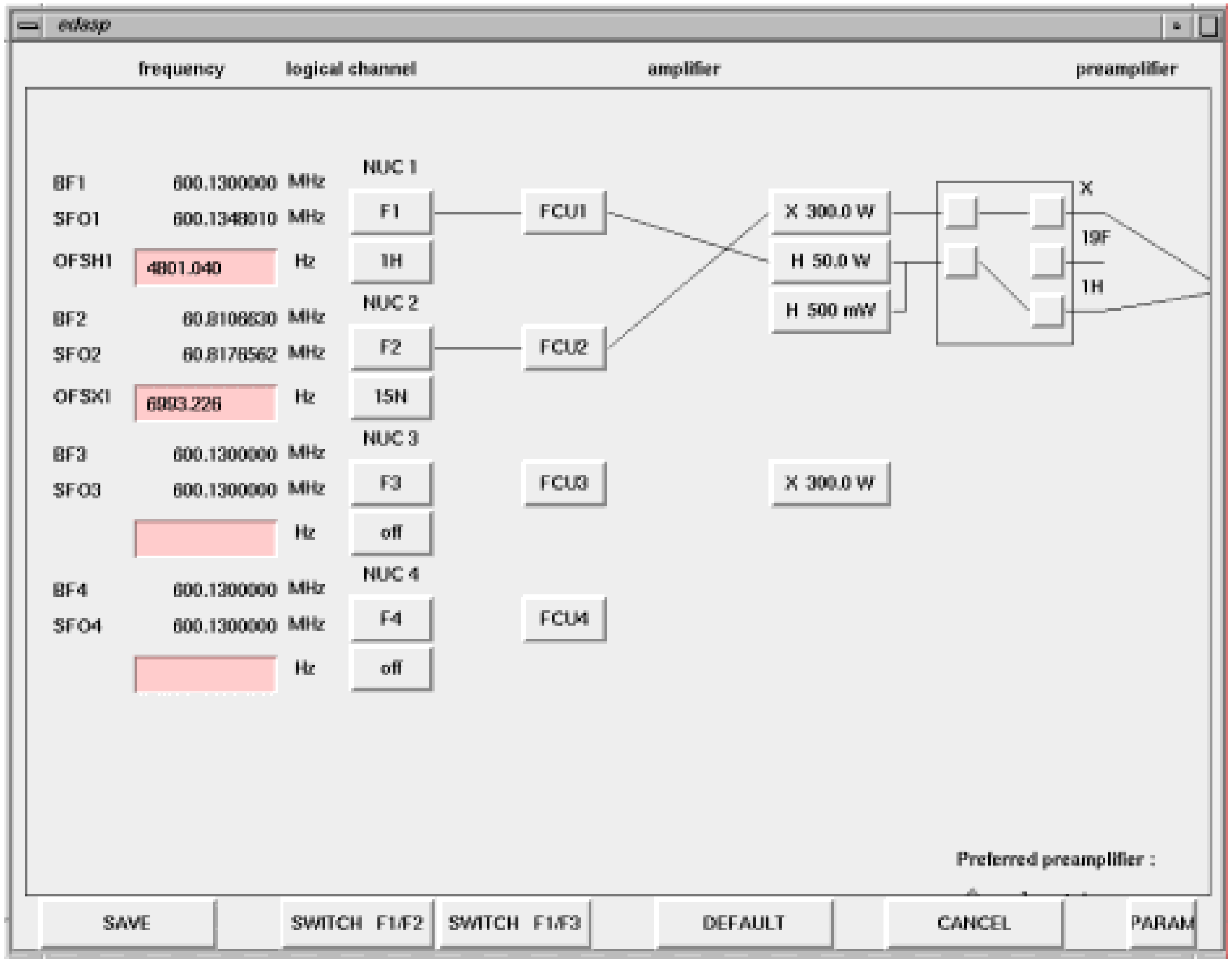
Users need to adjust parameters in "red" (meaning of the parameter in "green")

Set up hetero nuclear 2D/3D

(Bruker AV system)

Wobble all 3 channels: 15N→ 13C → 1H

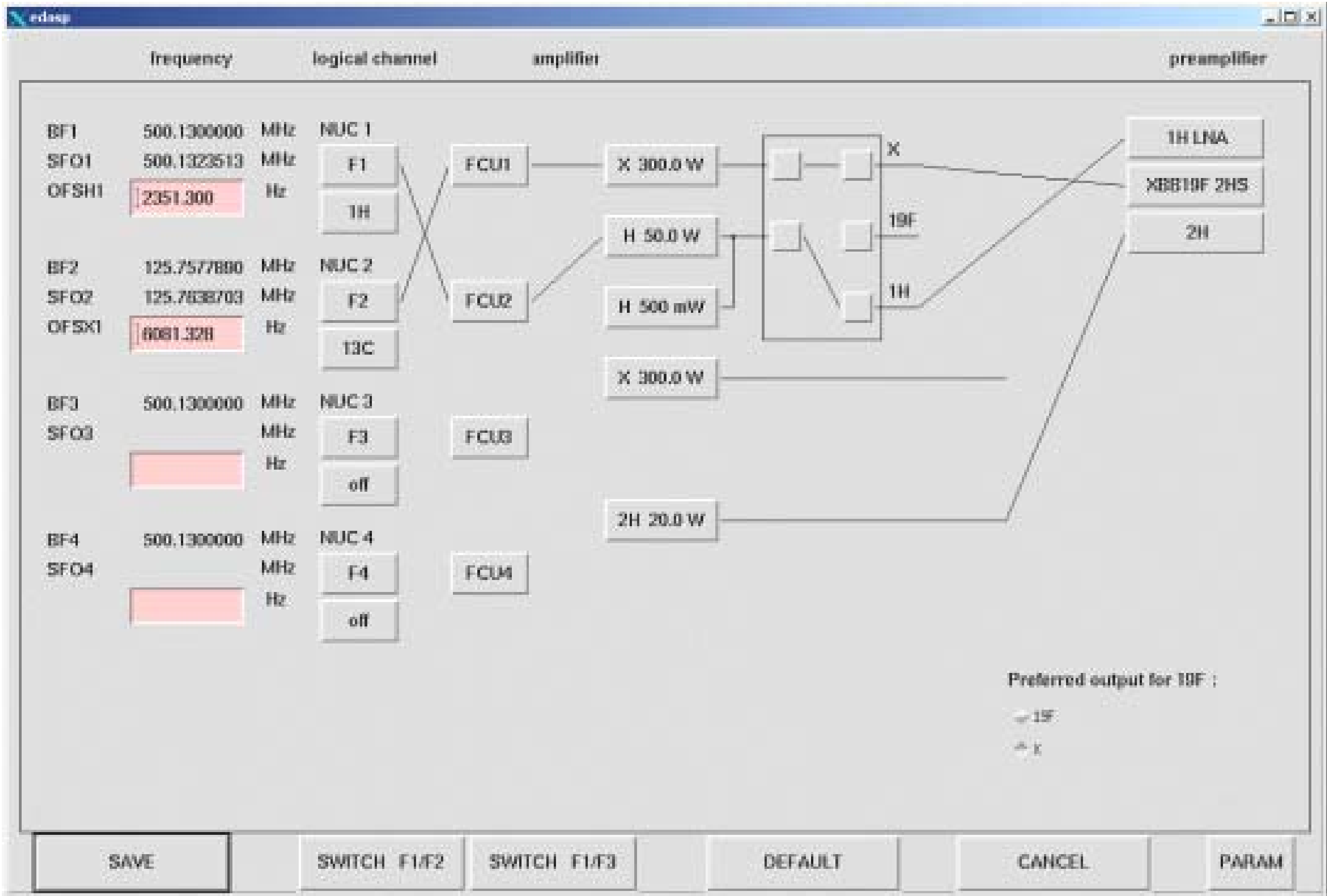
Step 1.1: (15N first) edasp to change setting and connection



Step 1.2: type wobble to wobble 15N, type stop after tune and match

Wobble all 3 channels: 15N→ 13C → 1H

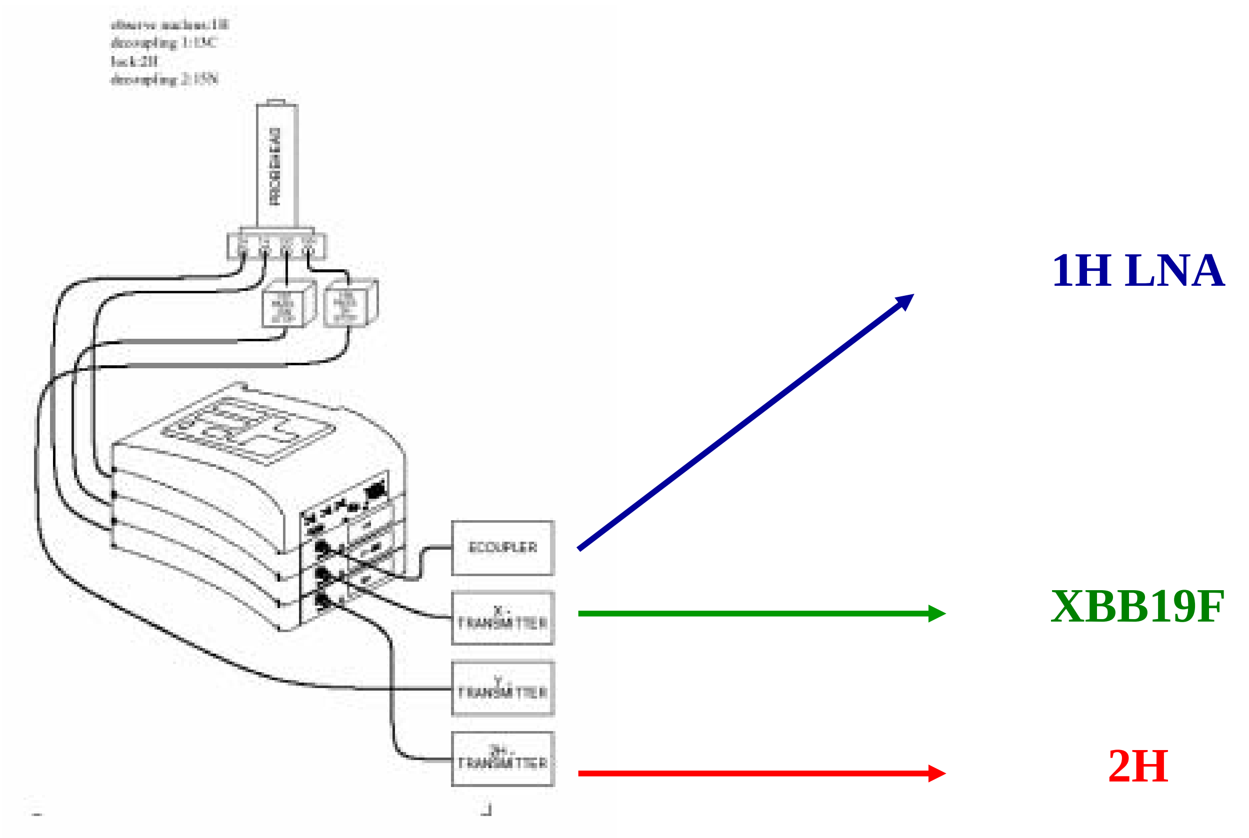
Step 2.1: (13C) edasp again to change setting and connection



Step 2.2: type wobble to wobble 13C,click any key on “HPPR” after 13C tuning and matching are done.

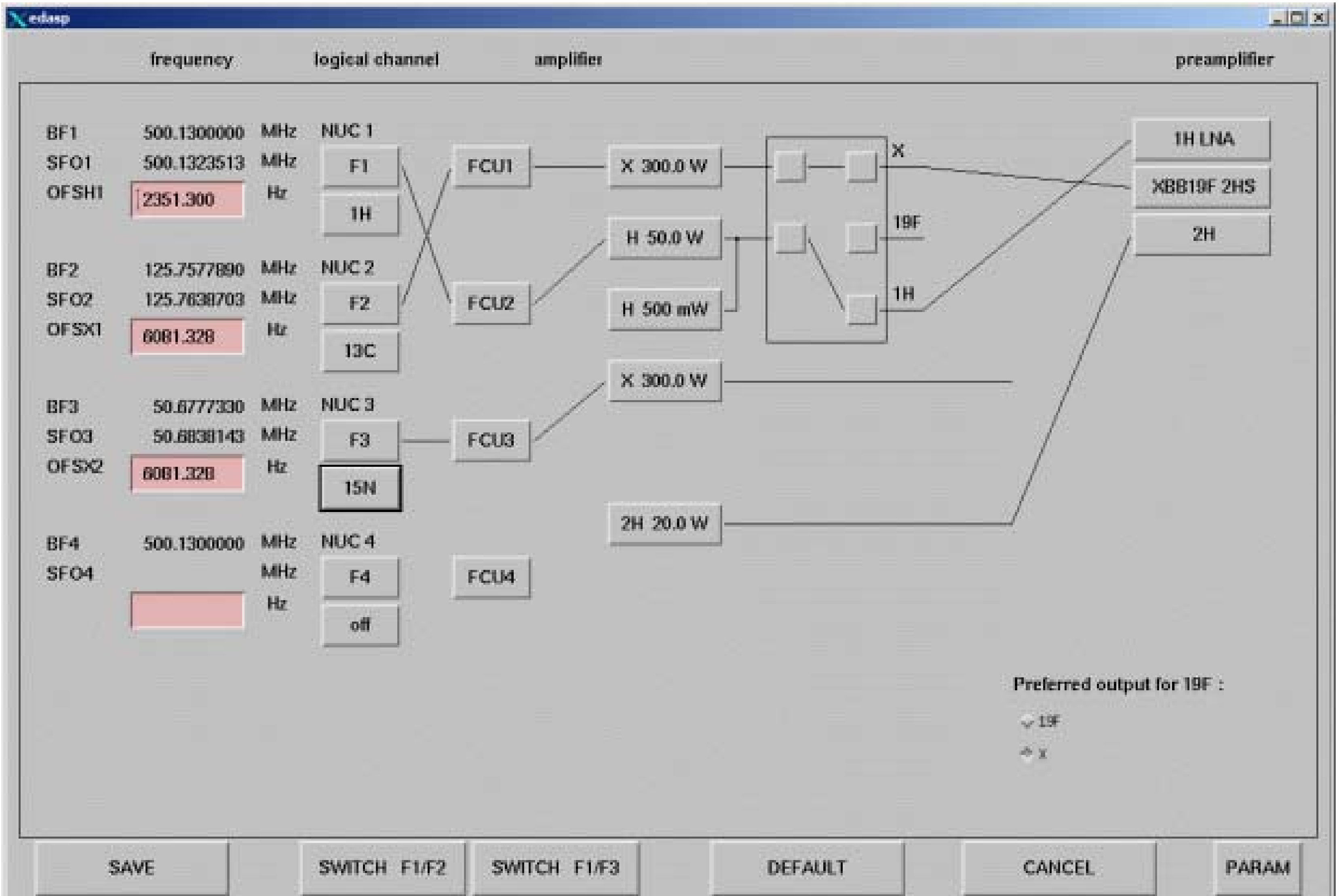
Wobble all 3 channels: 15N→ 13C → 1H

Step 3.1: click “Chn ↑“ on “HPPR” , then wait until wobble on 1H pop out (this might take 10-20 sec, please be patient!)



Step 3.2: type stop after tuning and matching are done for 1H.

Step 3.3: edasp to change setting and connection for the experiment



- **How to optimize condition? → For users: Follow Experiment Guide**

•**Experiment Name:** **2D 15N-1H HSQC**

•**Experiment Type:** **Using echo-antiecho, f1: H, f3:N**

•**Standard Parameter Set:** **std_2D_15N_HSQC_ET**

•**Pulse Program:** **invietf3gpsi**

•**AQ parameters to check**

1H pulses

pl1 (high power, ex: 0dB), p1 (90o pulse at pl1)

p28 (trim pulse, ex:1m)

Others

cnst4 (J H-N , ex: 90Hz)

d24 (1/4JH-N)

o1 (for 1H)

o3 (for 15N)

1 sw, 1td (for F1 dimension, N)

2 sw, 2 td (for F2 dimension, H)

d1

ns(=1*n)

ds(>=16)

rg

Users need to adjust parameters in "red" (meaning of the parameter in "green")

- **How to optimize condition? → For users: Follow Experiment Guide**

•**Experiment Name: 3D HNCO**

•**Experiment Type: Using Echo/antiecho , f1: H, f2:C, f3:N, F1(CO), F2(N), F3(H)**

•**Standard Parameter Set: std_3D_HNCO**

•**Pulse Program: hncogp3d.2**

•**AQ parameters to check**

1H pulses

pl1 (high power, ex: 0db), p1(90deg at pl1), p2(180deg at pl1)

pl19 (low power for dipsi2,pcpd1), p26(90deg at pl19), pcpd1(90deg ,ex: 40-50usec)

sp1 (shape pulse power for Sinc.1000) , p11(pulse length for sp1, ex: 2m)

Others

o1 (for 1H), o2 (for 13CO), o3 (for 15N)

1 sw, 1 td (for F1 dimension, ie: 13C)

2 sw, 2 td (for F2 dimension, ie:15N)

3 sw, 3 td (for F3 dimension, ie:1H)

d1, rg, ns(=8*n), ds (≧ 16)

Users need to adjust parameters in "red" (meaning of the parameter in "green")

- **How to optimize condition? → For operators : pulse program**

(1) hard pulse calibration for hetero nuclei

(2) Shape pulse calibration for hetero nuclei

(3) other "uniform" (sample independent) parameters set up

ex: delays, decouple program, gradient program, frequency jump..……

```
;hncogp3d.2
;avance-version (01/05/09)
;HNCO
…………( skips)
;     F1(H) -> F3(N) -> F2(C=O,t1) -> F3(N,t2) -> F1(H,t3)
;
;on/off resonance Ca and C=O pulses using shaped pulse
;phase sensitive (t1)
;phase sensitive using Echo/Antiecho gradient selection (t2)
…………( skips)
;sp1: f1 channel - shaped pulse  90 degree  (H2O on resonance)
;sp2: f2 channel - shaped pulse  90 degree  (C=O on resonance)
;sp3: f2 channel - shaped pulse 180 degree  (C=O on resonance)
;sp5: f2 channel - shaped pulse 180 degree  (Ca off resonance)
;sp8: f2 channel - shaped pulse  90 degree  (C=O on resonance)
;                 for time reversed pulse

…………( skips)
;d21: 1/(2J(NH)                       [5.5 msec]
;d23: 1/(4J(NCO)                      [12 msec]
;d26: 1/(4J'(NH)                      [2.3 msec]
…………( skips)
;cpds1: decoupling according to sequence defined by cpdprg1
;cpd3: decoupling according to sequence defined by cpdprg3
;pcpd1: f1 channel - 90 degree pulse for decoupling sequence
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence

;use gradient ratio:    gp 1 : gp 2 : gp 3 : gp 4 : gp 5
;                         60 :  -40 :   10 :   80 :  8.1
```

Thank you!!

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