2004 NMR User Training Course

High-Field Biomacromolecular Solution NMR Core Facility
National Research Program for Genomic Medicine

Date: September 06, 2004 (Monday)
Place: B1C Lecture Room, IBMS, Academia Sinica, Taipei
Title: Basic NMR Operation for Beginners

09:00-09:50	***Lecture 1: Basic NMR Concept and Facility Overview (Dr. Chi-Fon Chang, Facility Manager)***
10:00-10:50	***Lecture 2: Steps for NMR Experiments – small molecule (organics) (Dr. Casper Wu, Rezwave Application Scientist)***
11:00-11:50	***Lecture 3: Steps for NMR Experiments – larger molecule (biomolecules) (Dr. Wen-Jin Wu, Facility Staff Scientist)***
13:00-13:30	***Data Collection using Xwinmr (Dr. Chi-Fon Chang)***
13:30-14:00	***Brief Introduction on Xwinplot (Dr. Casper Wu)***
14:00 ---	***Hands On -- B2, IBMS or B1, CHEM (Tsun-Ai Yu, Pei-Ju Fang, Wen-Jin Wu, Casper Wu, Chi-Fon Chang)***

NMR Core Facility Overview

Chi-Fon Chang

09/06/2004

核心人員


Director 黃太煌
Operator 游春愛
Manager 張七鳳
Technician 李玉順
Operator 方珮如
Staff Scientist 吳文晉
Secretary 陳紅錦

核心設施—儀器設備

生醫所NMR Room

500MHz with CryoProbe

600MHz with CryoProbe

Computer Control

600MHz in 化學所

核心設施—儀器設備

not available yet

	NMR	System	Location	Remark
1	500 MHz (Bruker AV)	3 channels •TXI probe •Cryo probe	IBMS	Upgraded since Dec. 2002 Feb. 2004
2	600MHz (Bruker DRX)	3 channels •TXI & other probes	IBMS	Available since Aug.2002
3	600MHz (Bruker AV)	3 channels •BBO & TXI probes •TXI probe	CHEM	Available since Dec. 2002
4	600MHz (Bruker AV)	4 channels •QXI probe •Cryo Probe	IBMS	Available since Jan. 2003 March 2004
5	800MHz (Bruker AV)	4 channels •TXI Probe •Cryo Probe	IBMS	Available since July 2004 Dec. 2004

Service Items

- 一般服務

使用核心設施: 可由核心人員代測圖譜或自行操作取得圖譜

- 合作模式

共同研究: 由核心人員與使用者共同完成計劃

- 其他服務

- -定期訓練課程 (Users Training Courses)

- -Advance NMR Workshop (邀請專家學者)

核心公用電腦相關軟體

NMR data processing software

- **XWINNMR** *(process NMR data on IRIX 6.X & Linux)*
- **nmrPipe** *(process NMR data on IRIX6.X & Linux)*

NMR data analysis software

- **AURELIA** *(analyze NMR data on IRIX 6.X & Linux)*
- **nmrDraw** *(analyze NMR data on IRIX 6.X & Linux)*
- **nmrView** *(analyze NMR data on IRIX 6.X & Linux)*
- **Sparky** *(analyze NMR data on Linux)*
- **CARA** *(analyze NMR data on PC)*

Structure Calculation program and software

- **CSI Chemical Shift Index** *(making consensus plot on IRIX)*
- **TALOS** *(dihedral angles prediction on IRIX 6.X & Linux)*
- **XPLOR or CNS** *(structure calculation on IRIX & Linux)*
- **ARIA** *(auto NOE assign and structure calculation on IRIX6.X & Linux)*
- **CYANA** *(auto NOE assign and structure calculation on IRIX6.5 & Linux)*

NMR Core Facility

---User Information--

收費標準

2004.07.01修正

核磁共振儀	規格與單位	點數
Bruker 500SB	小時	45
Bruker 500SB Cryo probe	小時	60
Bruker 600US	小時	60
Bruker 600UB Cryo probe	小時	90
Bruker 800US2	小時	200
Bruker 800US2 Cryo probe	小時	265
大分子圖譜代測	實驗	1000
500MHz小分子低溫探頭圖譜代測	(每超過10分鐘加收20點)	30分鐘內完成之 每一實驗250點
600MHz小分子圖譜代測	(每超過10分鐘加收30點)	30分鐘內完成之 每一實驗300點
800MHz小分子圖譜代測	(每超過10分鐘加收45點)	30分鐘內完成之 每一實驗450點

- 每一點以新台幣一元計算，不足一小時以一小時計算
- 小分子圖譜代測30分鐘為最小單位，再依實際操作及資料處理時間計費

申請核心服務流程 (國科會)

作業流程

申請流程

相關表格及步驟

上網登錄意願表

(user) 登錄意願表

(core) 建立使用者帳戶

與核心洽談使用同意單內容

(user & core) 使用同意證明單四聯

依據使用同意書內容至科資中心繳費

(user) 領取收據

(user) 繳交四聯單及收據

(core) 將點數存入使用者帳戶

使用者帳戶需仍有”儲蓄”點數方可提供服務，點數不足者需再申購點數

核心服務流程

申請使用者帳戶(扣款帳戶)　申請表

取得使用者帳號(login 帳號)　申請表

預約儀器時間(網上或e-mail)　每月25日確認次月使用時間

蒐集圖譜(自行操作或核心代測)

數據傳送與確認簽收(網路或郵寄)　確認單 & 代測實驗記錄

其他服務與討論(相關軟體應用等)

網路預約

儀器可預約時間
(前一月25日方公布
最後確認使用時間)

填寫預約表

Send!
(核心收到後將
依序安排並回覆)

Temporary Reservation Only!! (目前預約狀況)

Final Schedule won't be available until 3/25. (最後時間分配表將於3/25公佈確認)

April 2004 [AVANCE 600 AV (IBMS)]

Thu	Fri	Sat	Sun	Mon	Tue	Wed
1	2	3	4	5 nitrogen	6	7
8	9	10	11	12 nitrogen	13	14
15	16 helium	17	18	19 nitrogen	20	21
22	23	24	25	26 nitrogen	27	28
29	30					

Today is : 19-Feb-2004 Fri
Last Updated : 08-Jan-2004 Thu

View Other Month Schedule : [1] | [current] | [3] | [4] | [5]

View Other SP Schedule : AV500 | DRX600 | AV600(IBMS) | AV600(CHEM) | AV800

schedule manager only | Time Reservation Home

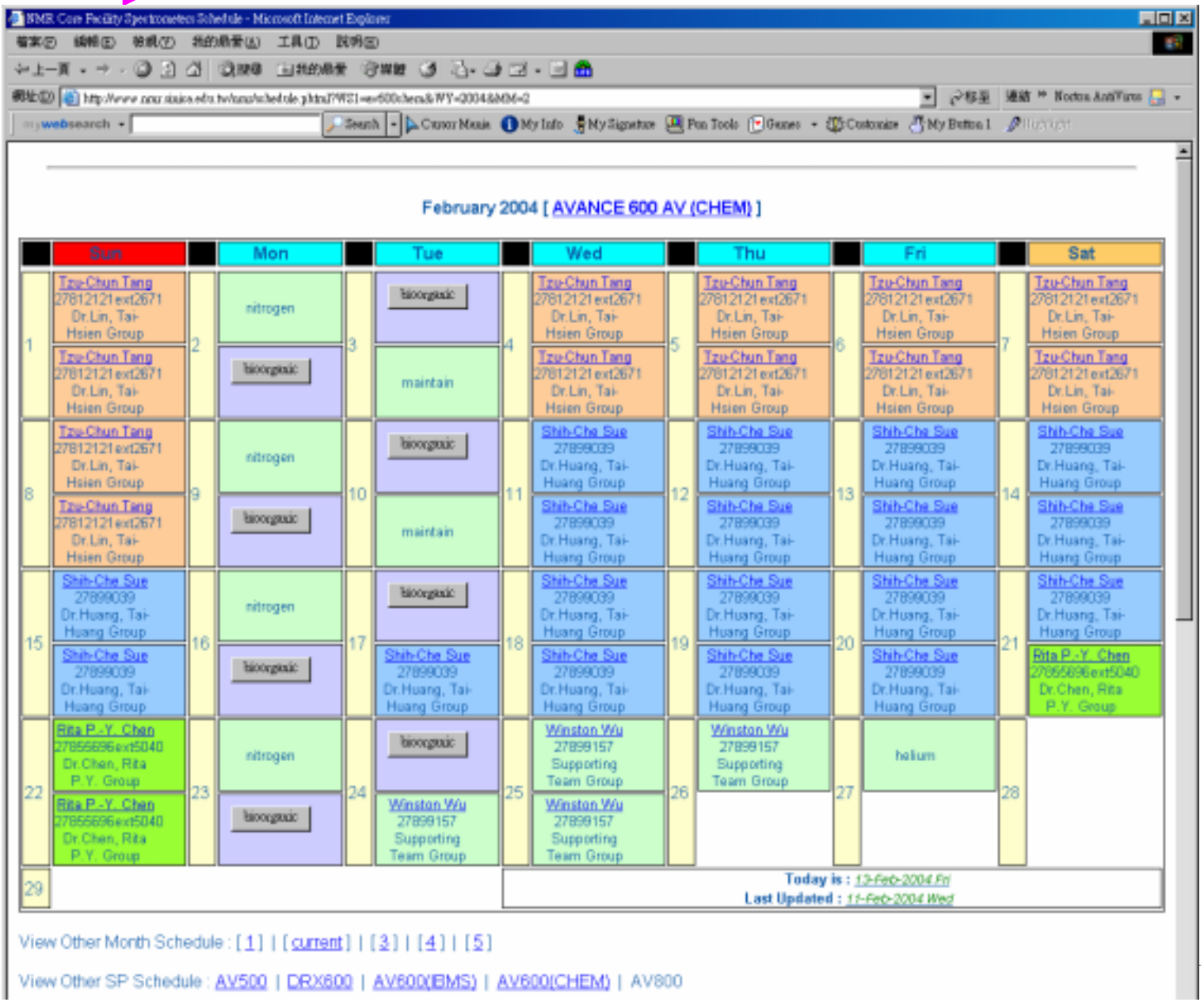
AVANCE 600 AV (IBMS) Reservation Form (暫用版 Temporary Form)

光譜儀 Spectrometer	AVANCE 600 AV (IBMS)
使用單位帳戶 Group Name	
使用者帳號 User Name	
使用者姓名 Full Name	
計畫主持人 PI	
連絡電話 Contact Phone no.	
E-mail	
預約時段 Preferred Start Time	Month : 2 DAY : 1
所需時間 Experiment Length	[eg. 7days,7hours]
操作方式 Operation Mode	○ 操作員 Operator ○ 自行操作 Self Operate
實驗名稱 Indicate Experiment	
其他需求 Others (若單「研究核心」人員協助進行新實驗或未經測試之實驗，請優先與「研究核心」人員討論，經「研究核心」人員測試無誤後，再預約。)	

Send | Reset

蒐集圖譜

核心將於前一月25日確認公布儀器分配表並e-mail 通知



February 2004 [AVANCE 600 AV (CHEM)]

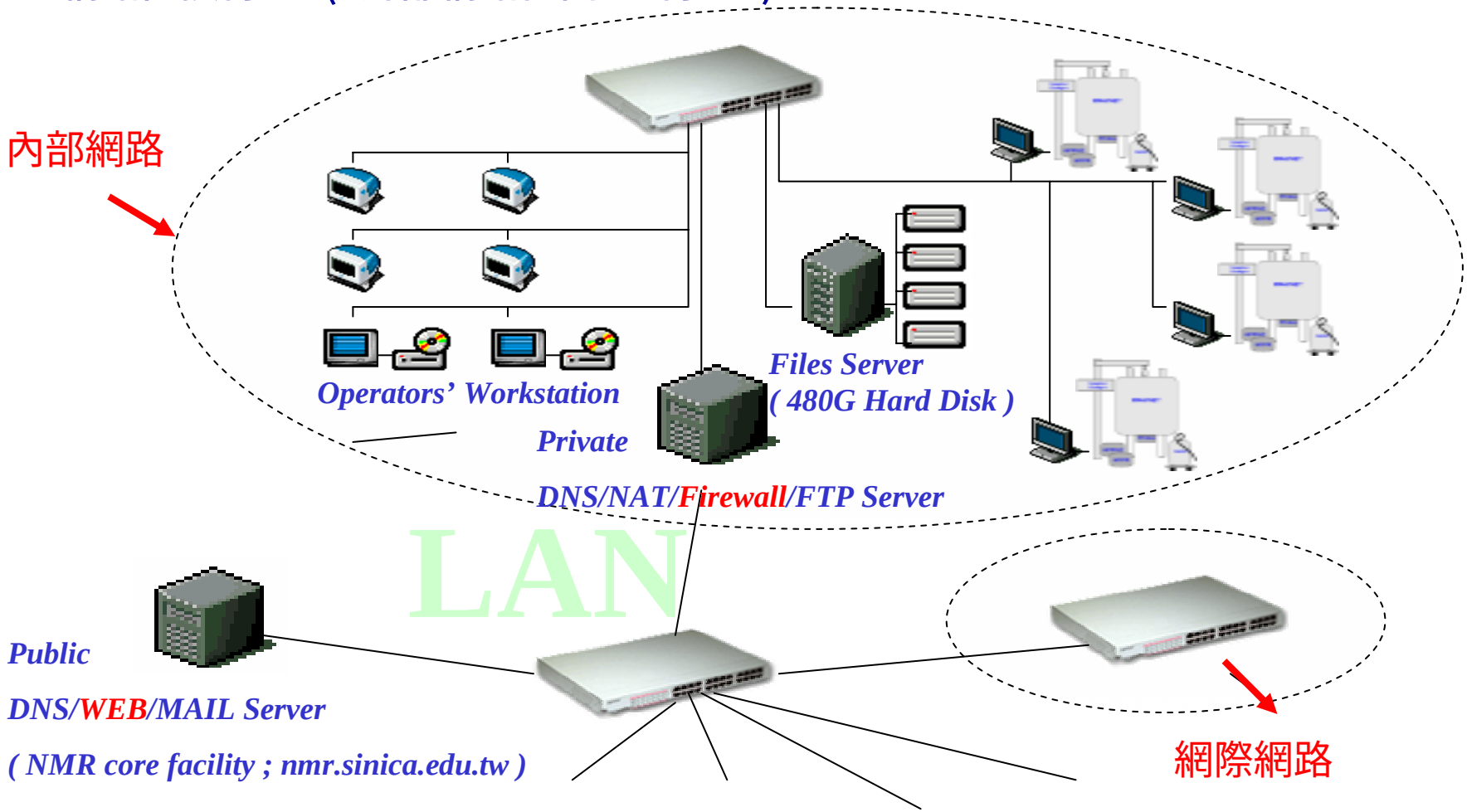
Sun	Mon	Tue	Wed	Thu	Fri	Sat
1 Tzu-Chun Tang 27812121 ext2671 Dr.Lin, Tai-Hsien Group Tzu-Chun Tang 27812121 ext2671 Dr.Lin, Tai-Hsien Group	2 nitrogen biorganic	3 biorganic maintain	4 Tzu-Chun Tang 27812121 ext2671 Dr.Lin, Tai-Hsien Group Tzu-Chun Tang 27812121 ext2671 Dr.Lin, Tai-Hsien Group	5 Tzu-Chun Tang 27812121 ext2671 Dr.Lin, Tai-Hsien Group Tzu-Chun Tang 27812121 ext2671 Dr.Lin, Tai-Hsien Group	6 Tzu-Chun Tang 27812121 ext2671 Dr.Lin, Tai-Hsien Group Tzu-Chun Tang 27812121 ext2671 Dr.Lin, Tai-Hsien Group	7 Tzu-Chun Tang 27812121 ext2671 Dr.Lin, Tai-Hsien Group Tzu-Chun Tang 27812121 ext2671 Dr.Lin, Tai-Hsien Group
8 Tzu-Chun Tang 27812121 ext2671 Dr.Lin, Tai-Hsien Group Tzu-Chun Tang 27812121 ext2671 Dr.Lin, Tai-Hsien Group	9 nitrogen biorganic	10 biorganic maintain	11 Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group	12 Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group	13 Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group	14 Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group
15 Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group	16 nitrogen biorganic	17 biorganic Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group	18 Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group	19 Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group	20 Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group	21 Shih-Che Sue 27899039 Dr.Huang, Tai-Huang Group Rita P.-Y. Chen 27856696 ext5040 Dr.Chen, Rita P.Y. Group
22 Rita P.-Y. Chen 27856696 ext5040 Dr.Chen, Rita P.Y. Group Rita P.-Y. Chen 27856696 ext5040 Dr.Chen, Rita P.Y. Group	23 nitrogen biorganic	24 biorganic Winston Wu 27899157 Supporting Team Group	25 Winston Wu 27899157 Supporting Team Group Winston Wu 27899157 Supporting Team Group	26 Winston Wu 27899157 Supporting Team Group	27 helium	28
29			Today is : 13-Feb-2004 Fri Last Updated : 11-Feb-2004 Wed			

View Other Month Schedule : [1] | [current] | [3] | [4] | [5]

View Other SP Schedule : AV500 | DRX600 | AV600(BMS) | AV600(CHEM) | AV800

資料傳送

1. 自取或郵寄：光碟片,磁片,或抽取式硬碟……
2. 網路直接傳送（ 外部網路僅由FTP傳送 ）



核心資訊網站
http://www.nmr.sinica.edu.tw/

Basic NMR Concepts

Chi-Fon Chang

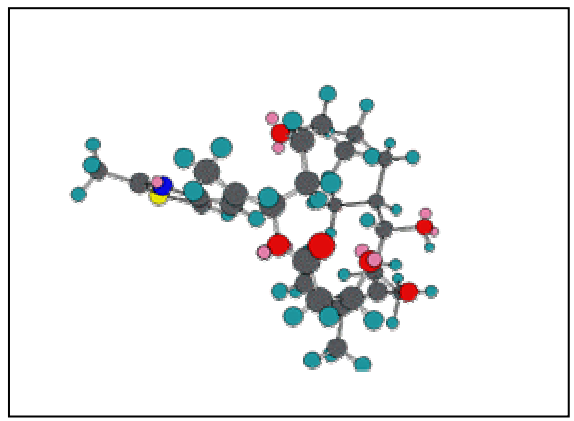
09/06/2004

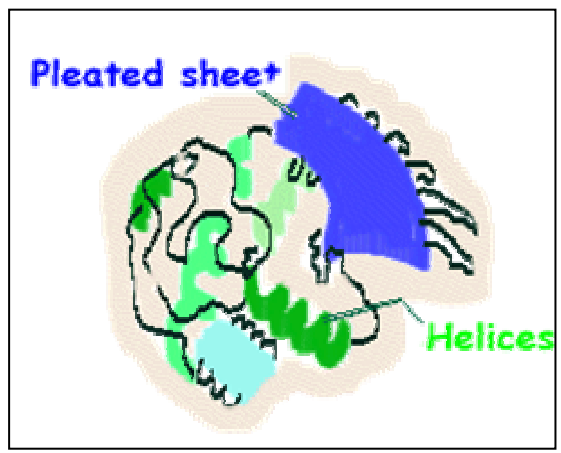
The problem the we want to solve

What we "really" see

How ?

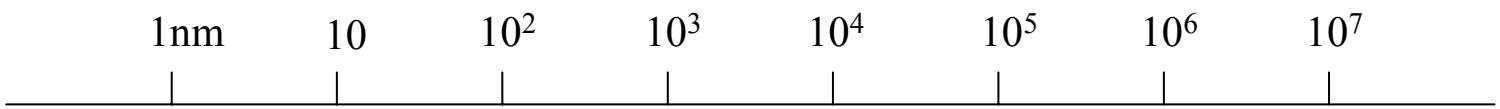
What we want to "see"





How about Spectroscopy?

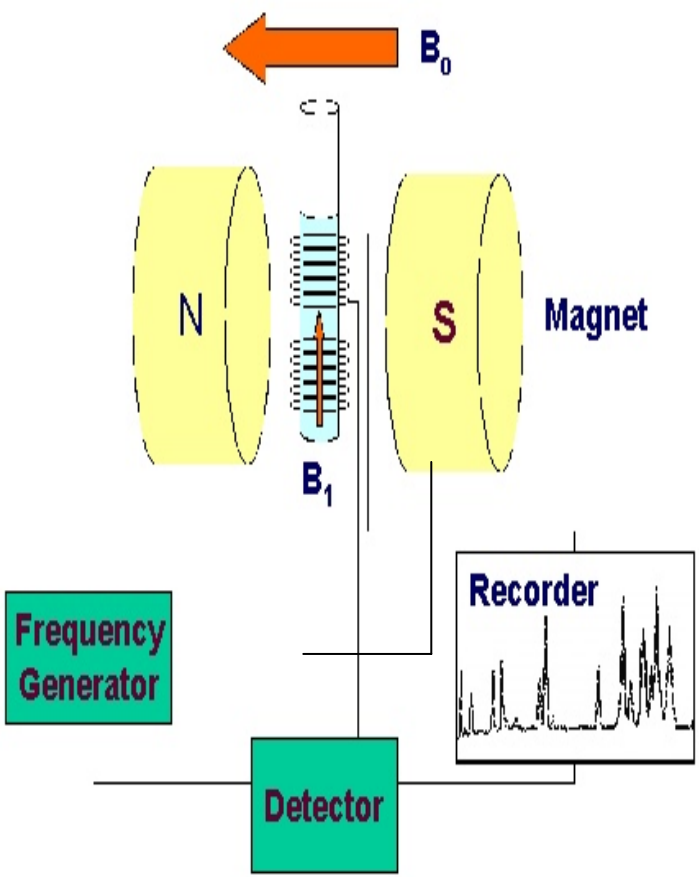
Spectroscopy



(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**
		CD	**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)

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 $-I, -I+1, \ldots..+I$.
(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 , $\gamma = 267.512 *10^6$ rad $T^{-1}sec^{-1}$

13C: I=1/2 , $\gamma = 67.264*10^6$

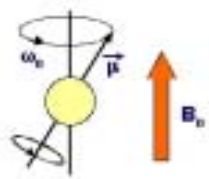
15N: I=1/2 , $\gamma = 27.107*10^6$

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 ^1H process at a frequency of 600.13MHz? What would be the process frequency for ^{13}C at the same field?

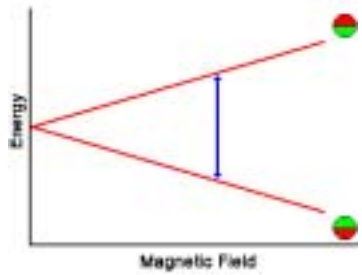
◆ The Nucleus in a Magnetic Field B_0

B_0 (the magnet of machine)

(1) Provide energy for the nuclei to spin

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

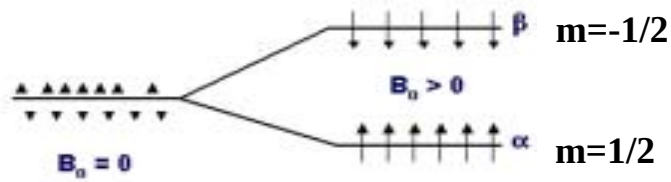
Larmor precession $\upsilon = \omega/2\pi = \gamma B_0/2\pi$



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

The stronger the magnetic field B_0, the greater separation

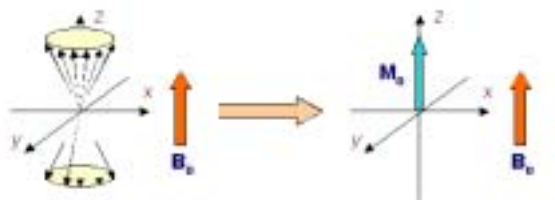
$P_{m=-1/2} / P_{m=+1/2} = e^{-\Delta E/kT}$



(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)

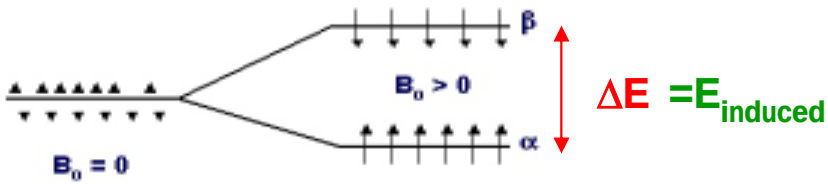


◆ When the Nucleus Meet the "right" Magnet: N. M. Resonance

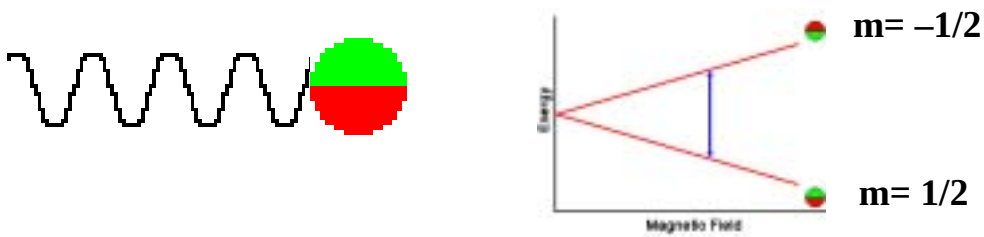
B_1(the irradiation magnet, current induced)

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

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



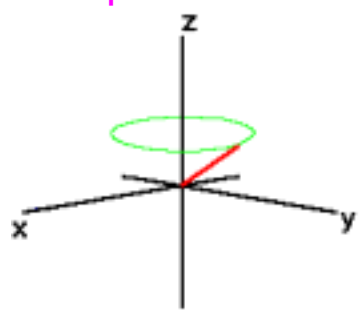
And now, the spin jump to the higher energy (from m=1/2→m= – 1/2)



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

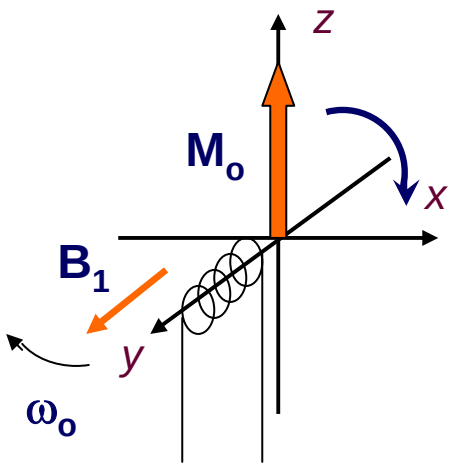
$M_z=M\cos\alpha$

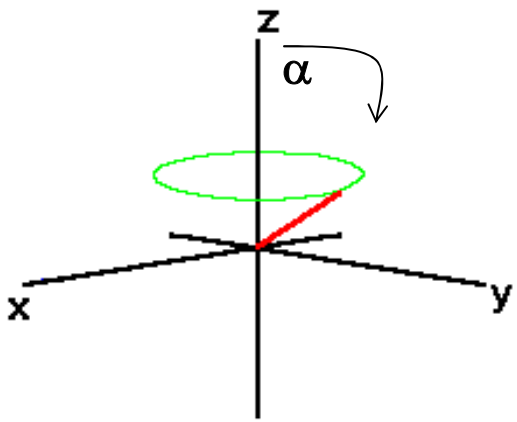
$M_{xy}=M\sin\alpha$.



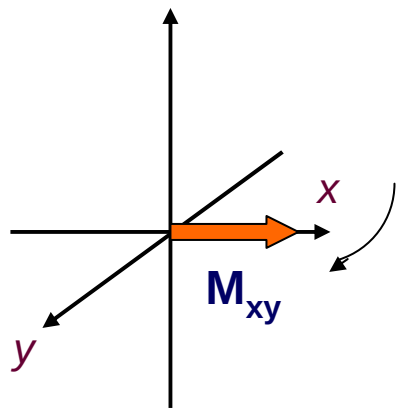
What happen during irradiation

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 90° pulse !!

What happen after irradiation ceases

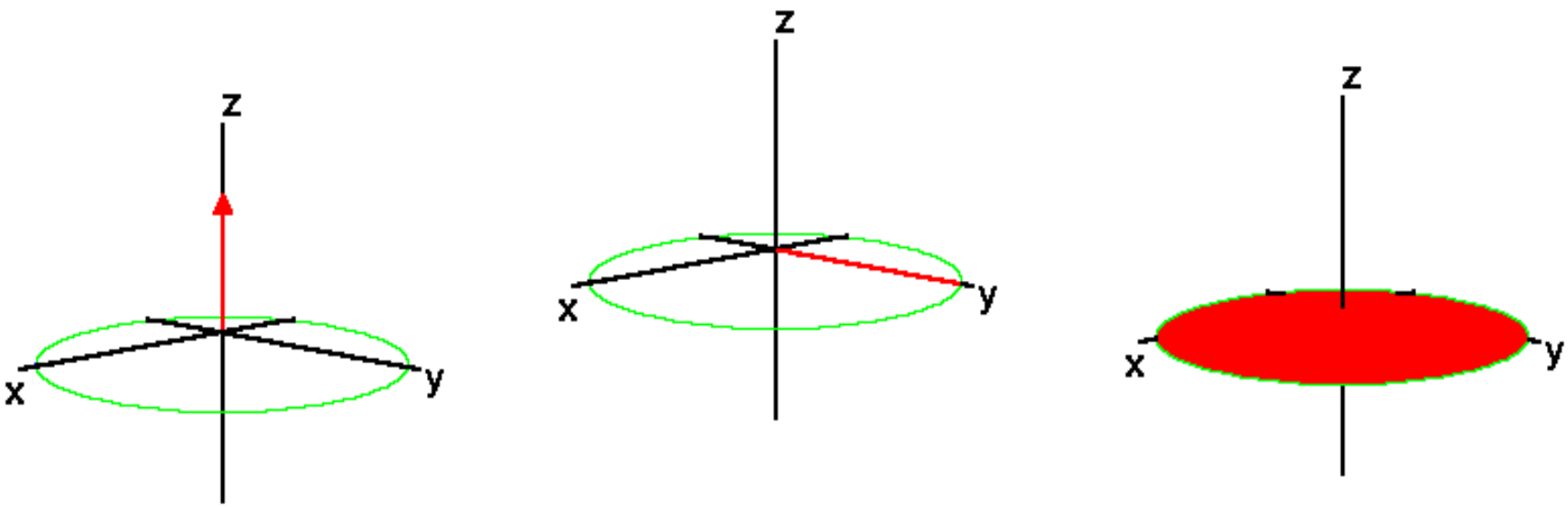
•After irradiation ceases, not only do the population of the states revert to a Boltzmann distribution, but also the individual nuclear magnetic moments begin to lose their phase coherence and return to a random arrangement around the z axis.

(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)

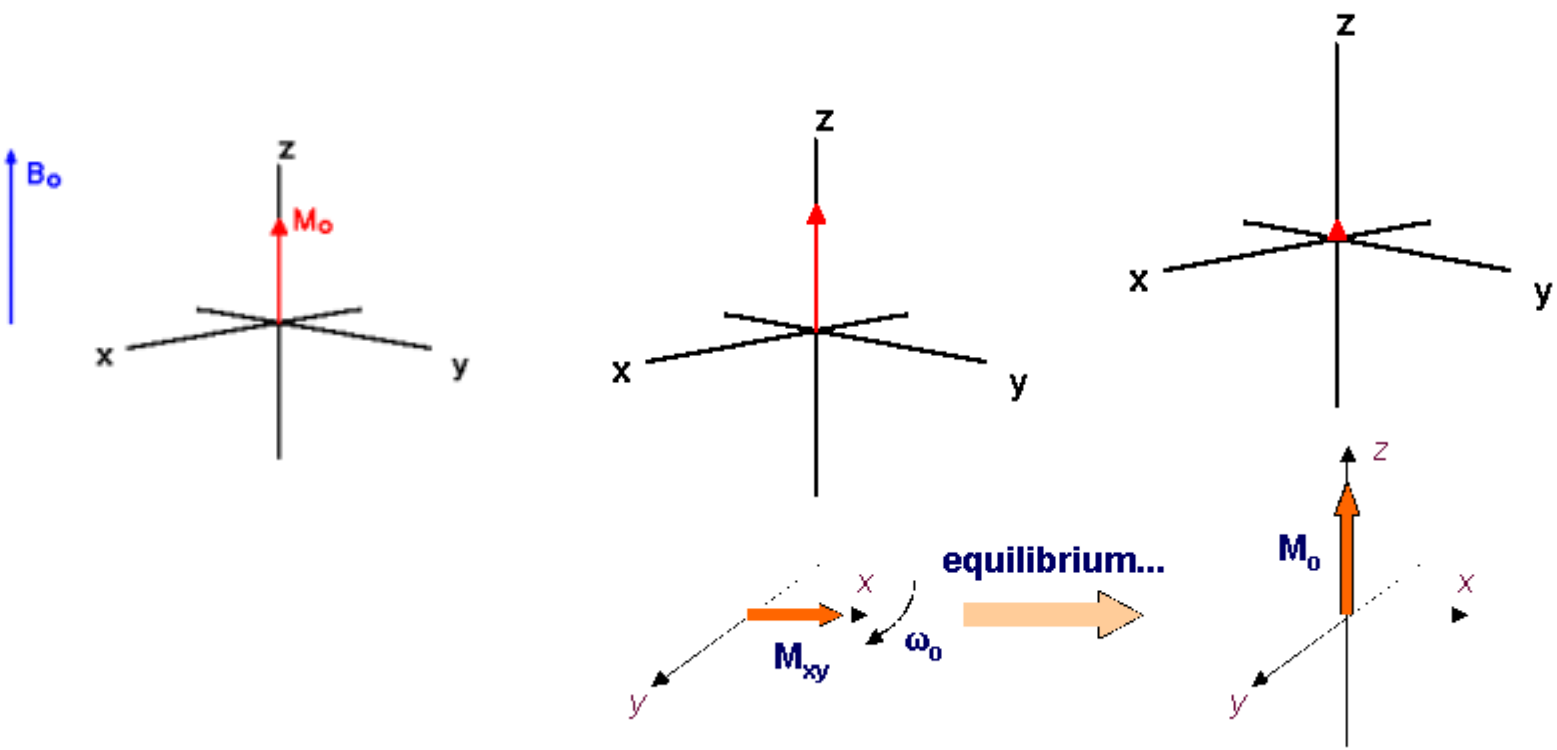
• How long after immersion in a external field does it take for a collection of nuclei to reach Boltzmann distribution is controlled by T1, the spin lattice relaxation time.

(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)

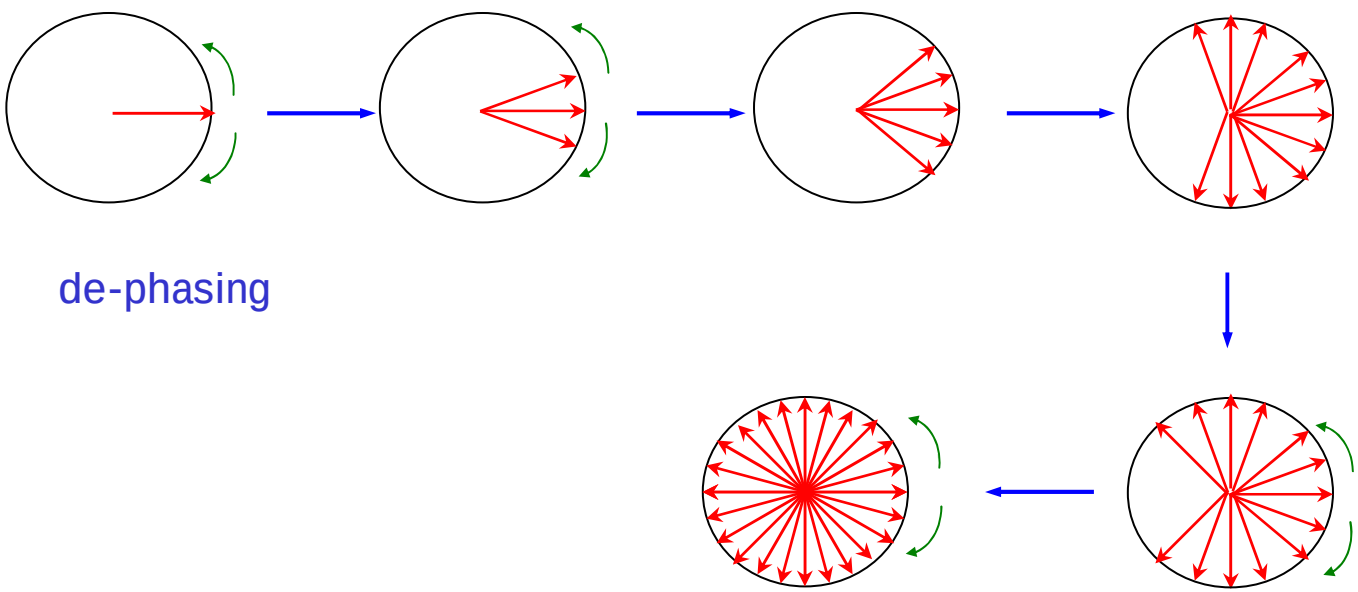
• This process for nuclei begin to lose their phase coherence and return to a random arrangement around the z axis is called spin-spin relaxation.

(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.

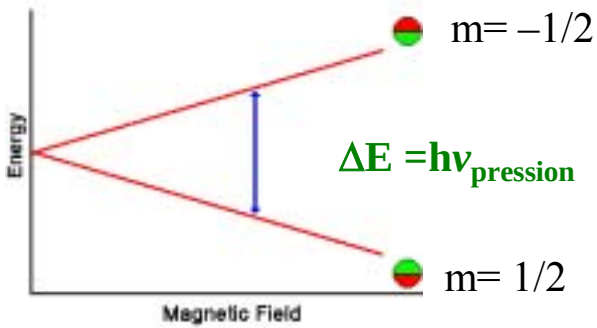
$dM_x/dt=-M_x/T2$

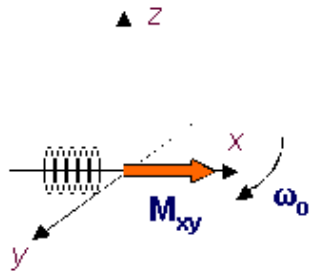
$dM_y/dt=-M_y/T2$



◆ Collecting NMR signals

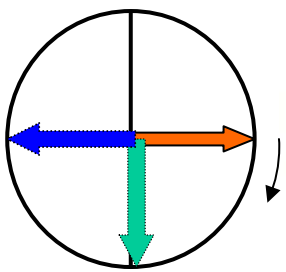
•The detection of NMR signal is on the xy plane. The oscillation of Mxy generate a current in a coil , which is the NMR signal.

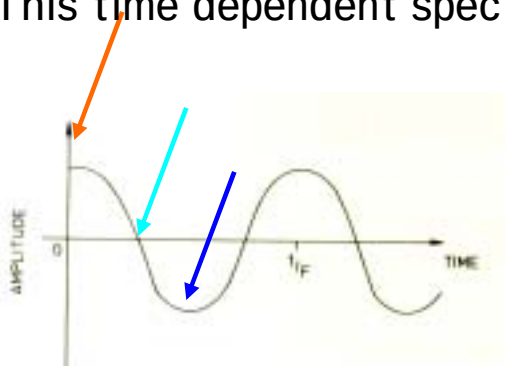




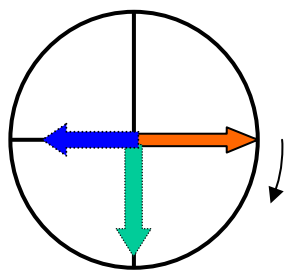
Receiver coil (x) ⇒ NMR signal

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

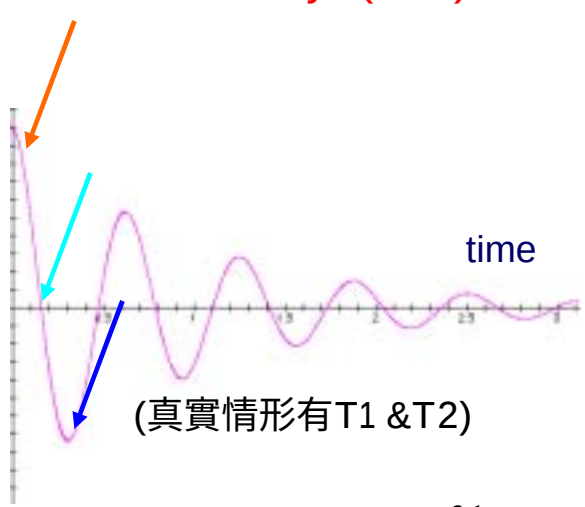




(如果沒有 relaxation)



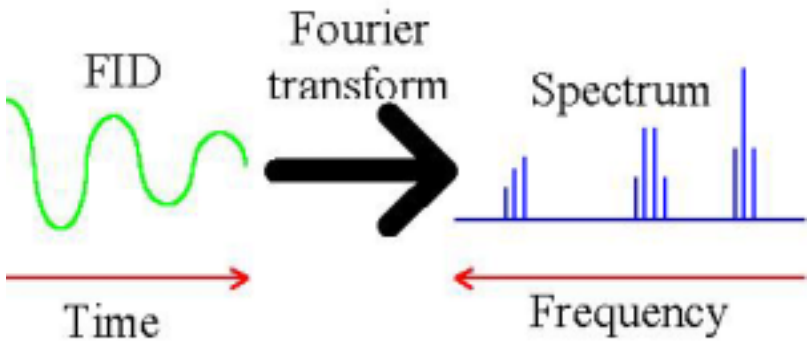
M_{xy}

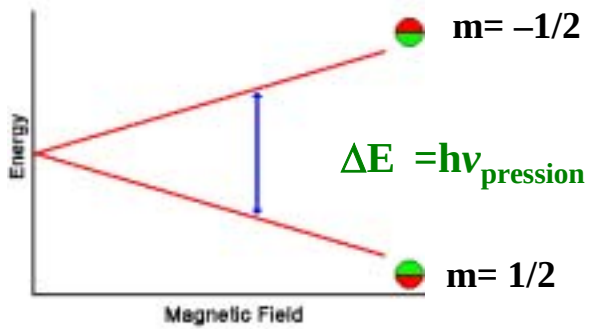


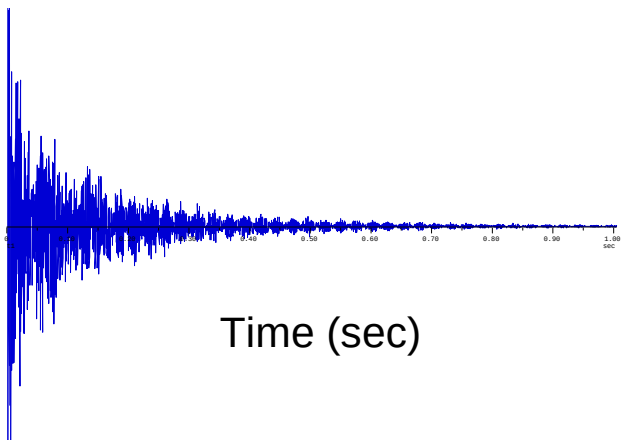
(真實情形有T1 &T2)

NMR Data Processing

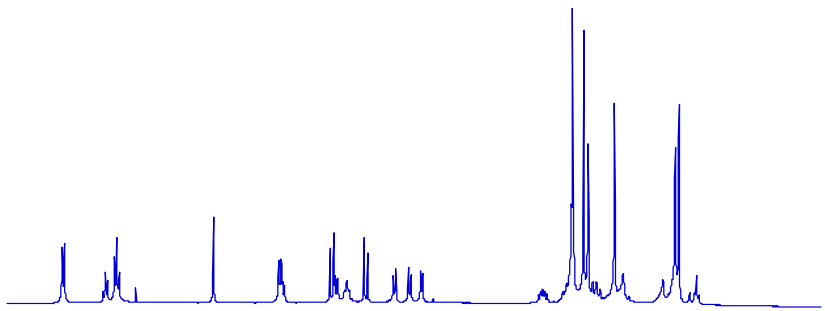
- The FID (free induction decay) is then Fourier transform to frequency domain to obtain $\upsilon_{pression}$ (chemical shift) for each different nuclei.







Time (sec)



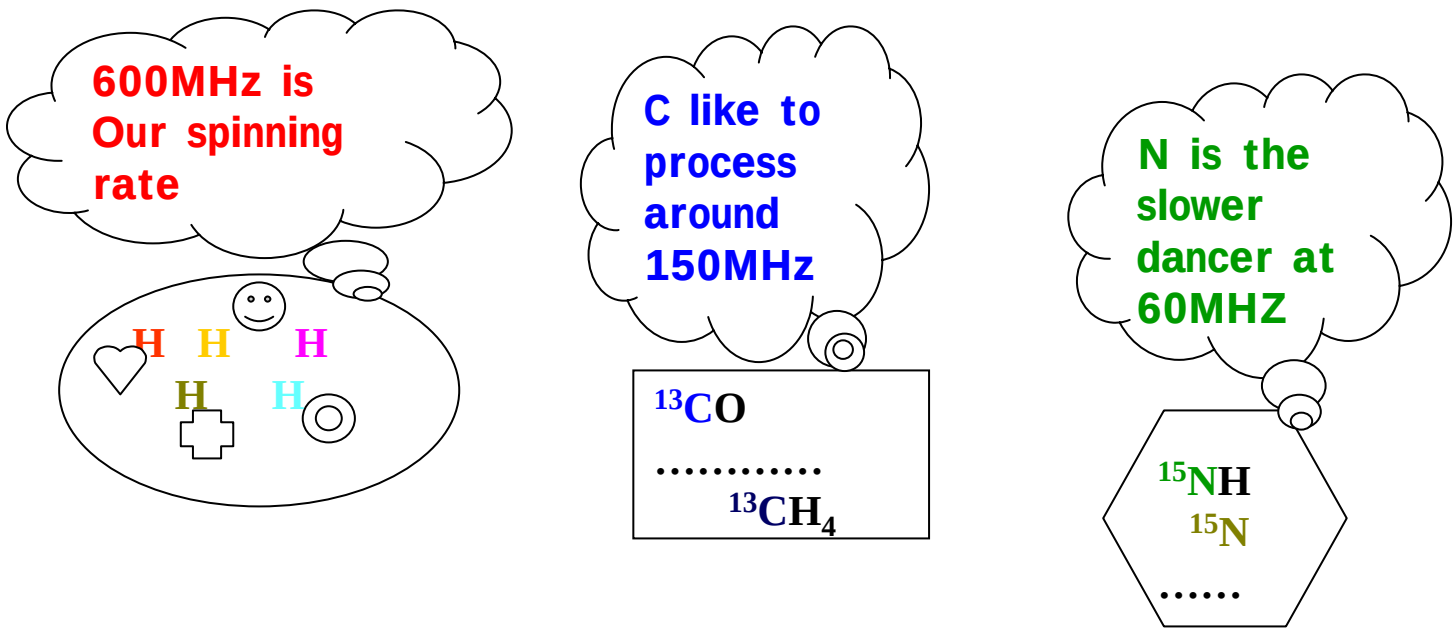
Frequency (Hz)

Basic of NMR signal assignment

- 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.

< At 14.7 Tesla >



Basic of NMR signal assignment

- 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

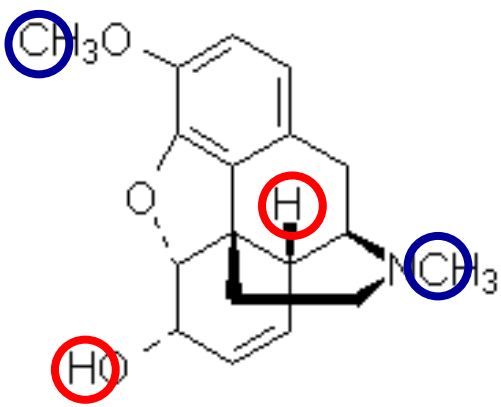
$B_{eff}=(1-\sigma)\ B_0$

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

$\sigma = 0$ → naked nuclei

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

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



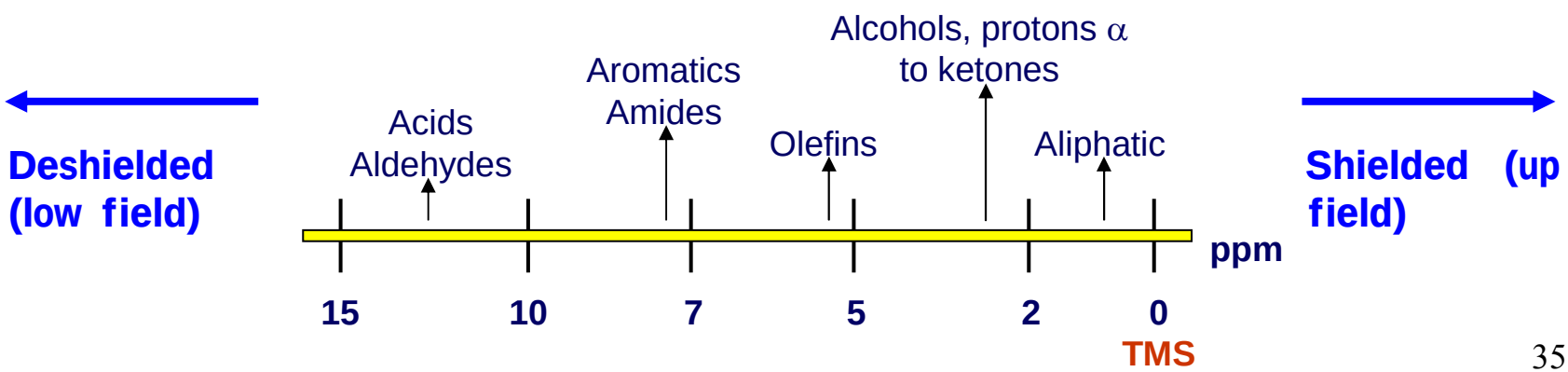
Chemical Shift

- The chemical shift of a nucleus is the difference between the resonance frequency of the nucleus and a standard, relative to the standard. This quantity is reported in ppm and given the symbol delta,

$$\delta = (\nu - \nu_{REF}) \times 10^6 / \nu_{REF}$$

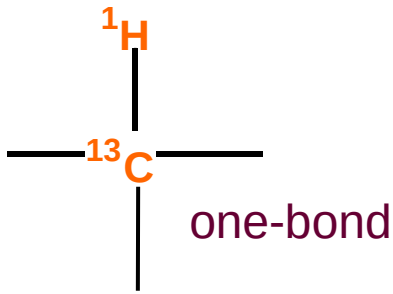
- In 1H NMR spectroscopy, this standard is often tetramethylsilane, $Si(CH_3)_4$, abbreviated TMS, or 2,2-dimethyl-2-silapentane-5-sulfonate, DSS, in biomolecular NMR.

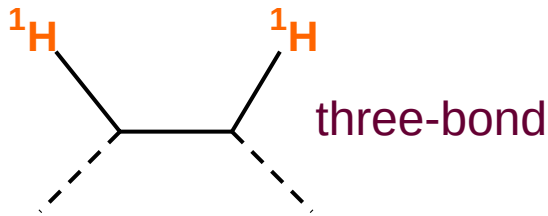
- The good thing is that since it is a relative scale, the δ for a sample in a 100 MHz magnet (2.35 T) is the same as that obtained in a 600 MHz magnet (14.1 T).



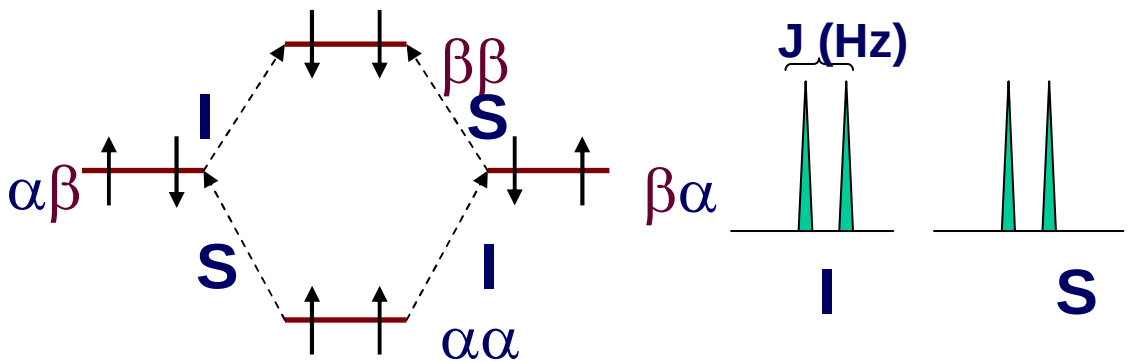
J-coupling

•Nuclei which are close to one another could cause an influence on each other's effective magnetic field. If the distance between non-equivalent nuclei is less than or equal to three bond lengths, this effect is observable. This is called spin-spin coupling or J coupling.





•Each spin now seems to has two energy 'sub-levels' depending on the state of the spin it is coupled to:



The magnitude of the separation is called *coupling constant* (J) and has units of Hz.

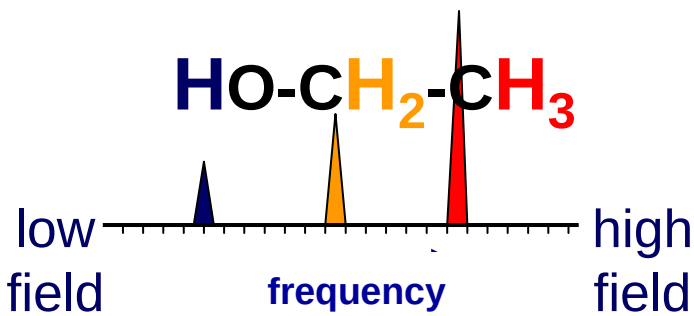
Basic of Assignment

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

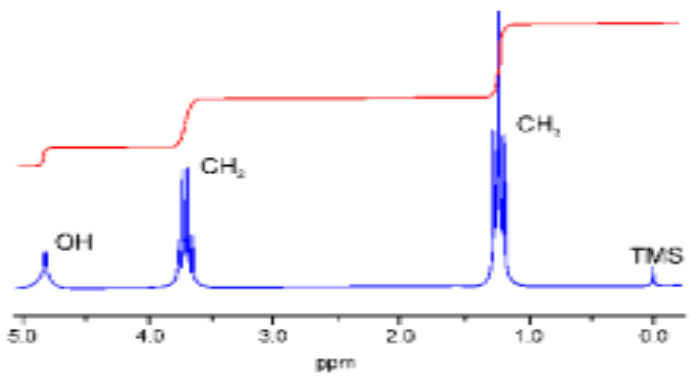
$\sigma = 0$ → naked nuclei

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

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



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



Steps for NMR Experiment

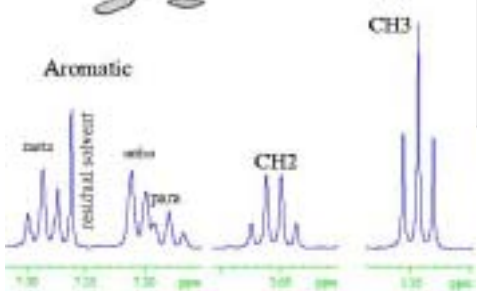
取得樣品 → 取得ＮＭＲ圖譜 → 分析圖譜結果

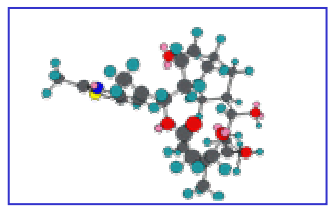
適當的實驗方法

標定ＮＭＲ譜線

鑑定化學(生化)分子

分子結構、動力學、反應機制……

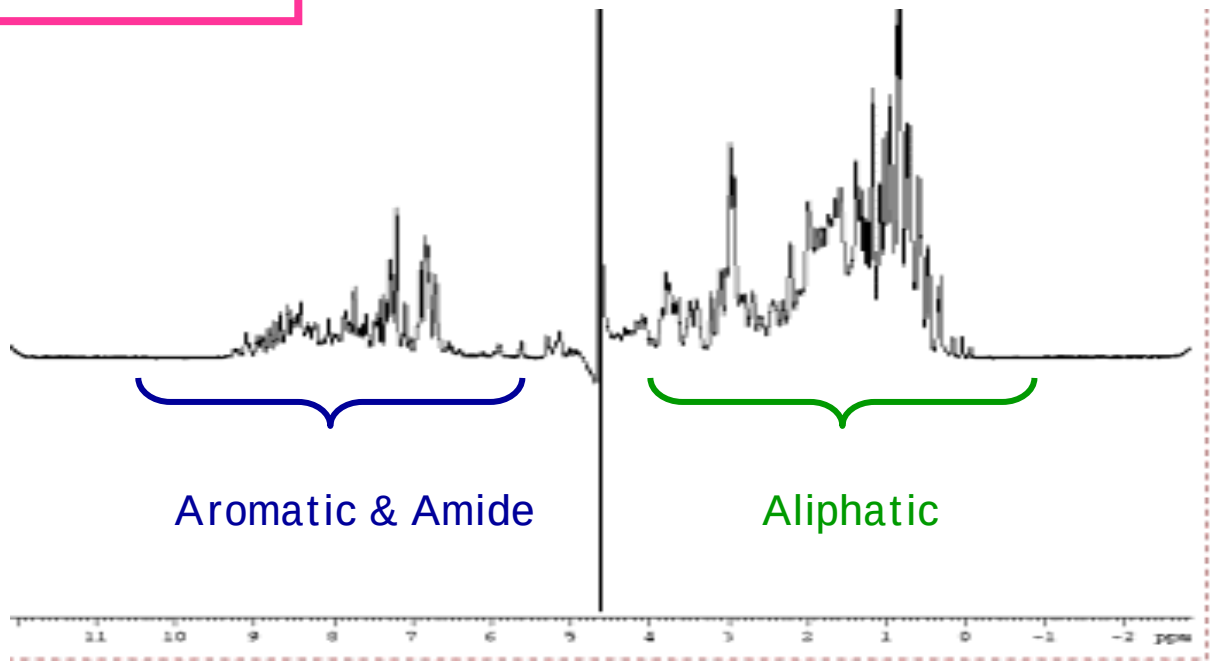


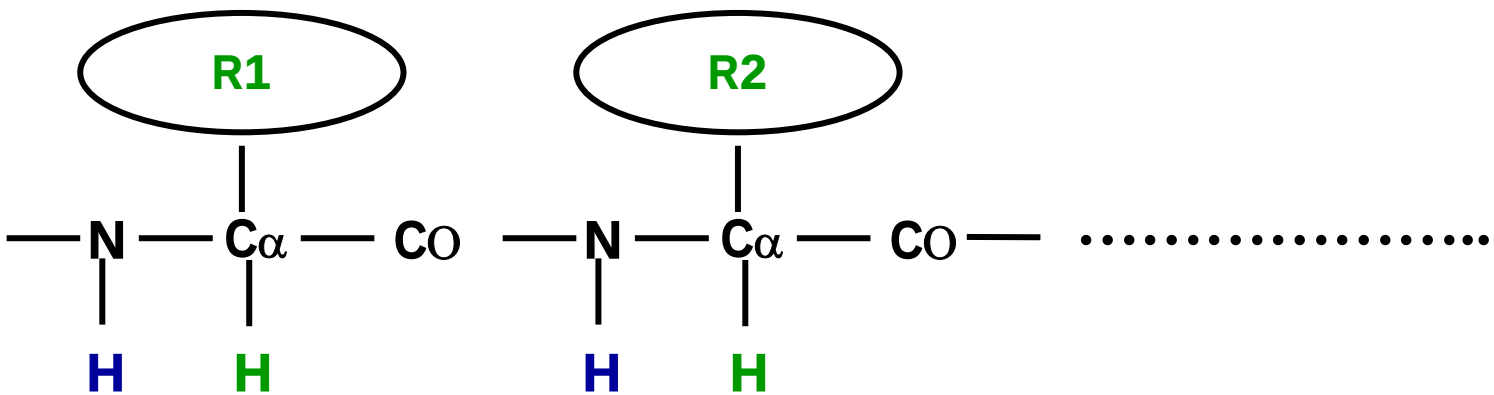


Introduction of NMR Experiments

Homo Nuclear 1D NMR

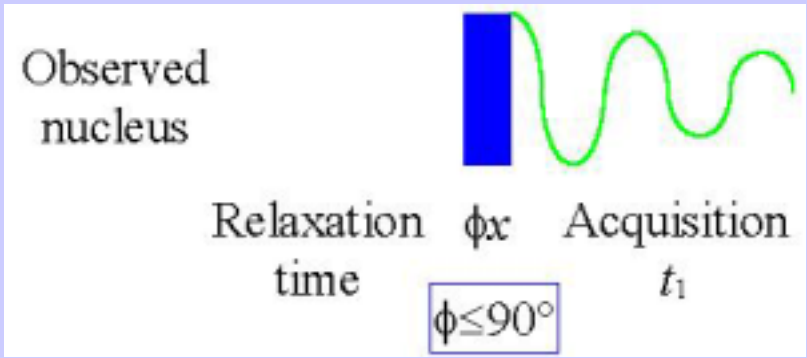
1D one pulse 1H

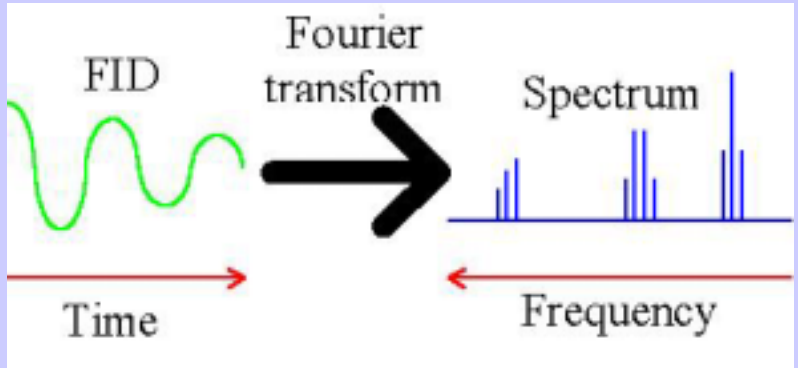




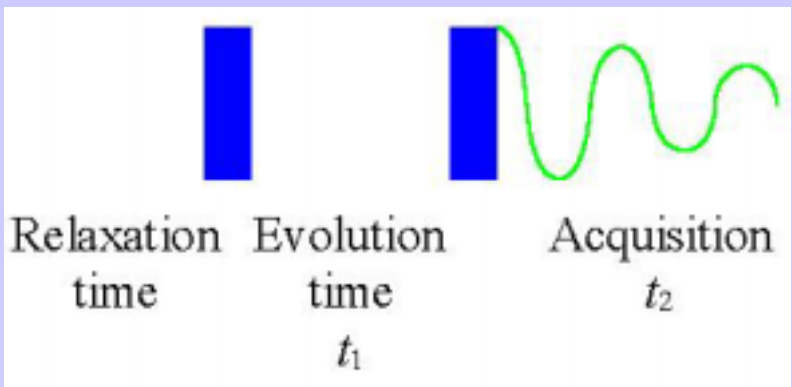
Homo/Hetro Nuclear 2D NMR

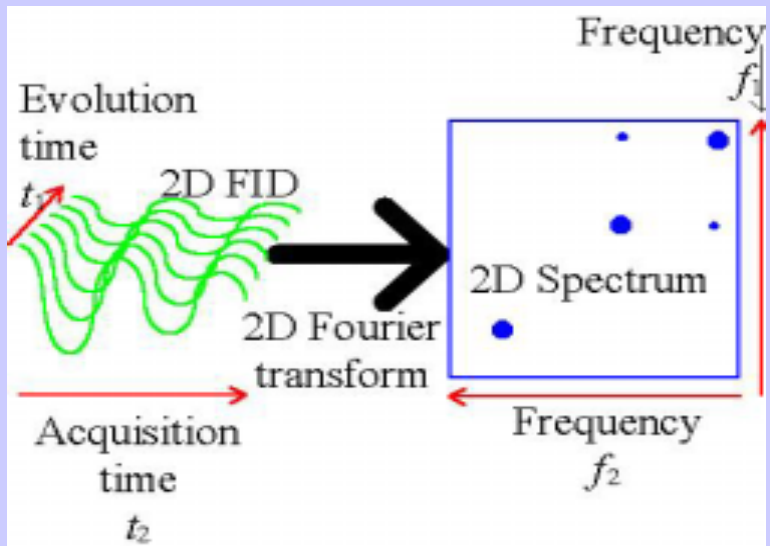
Basic 1D Experiment





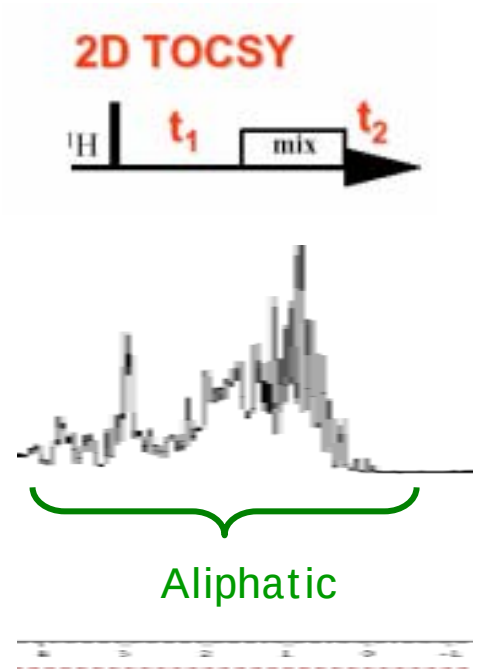
Basic 2D Experiment

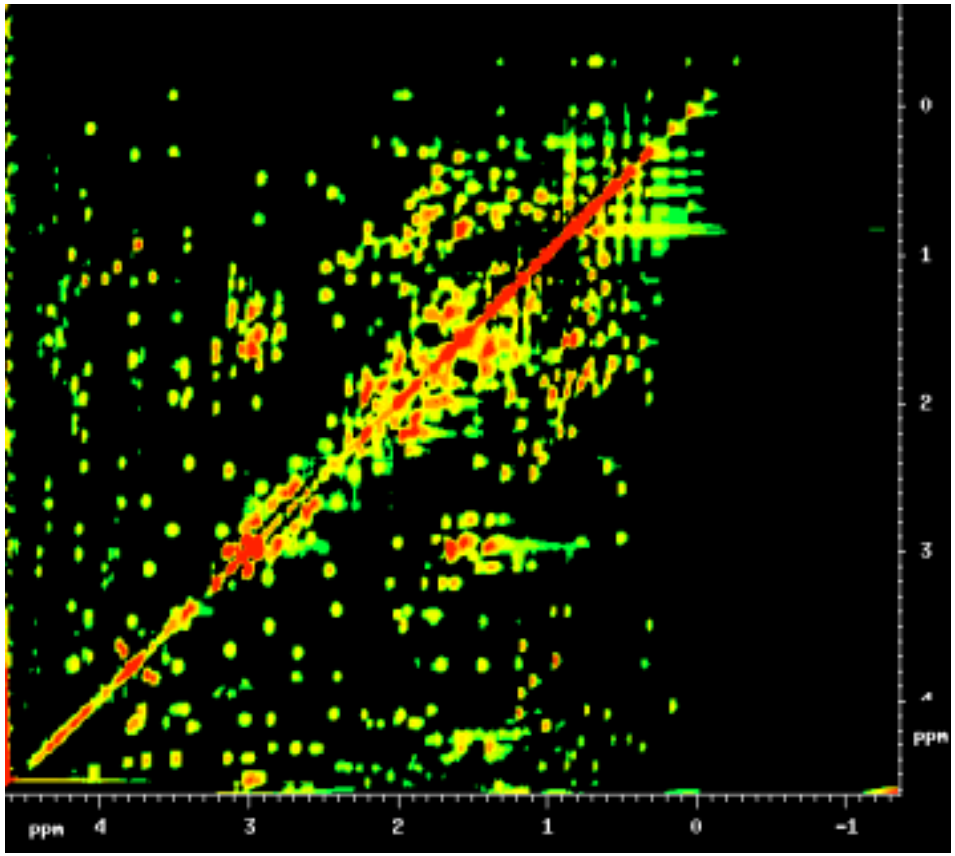


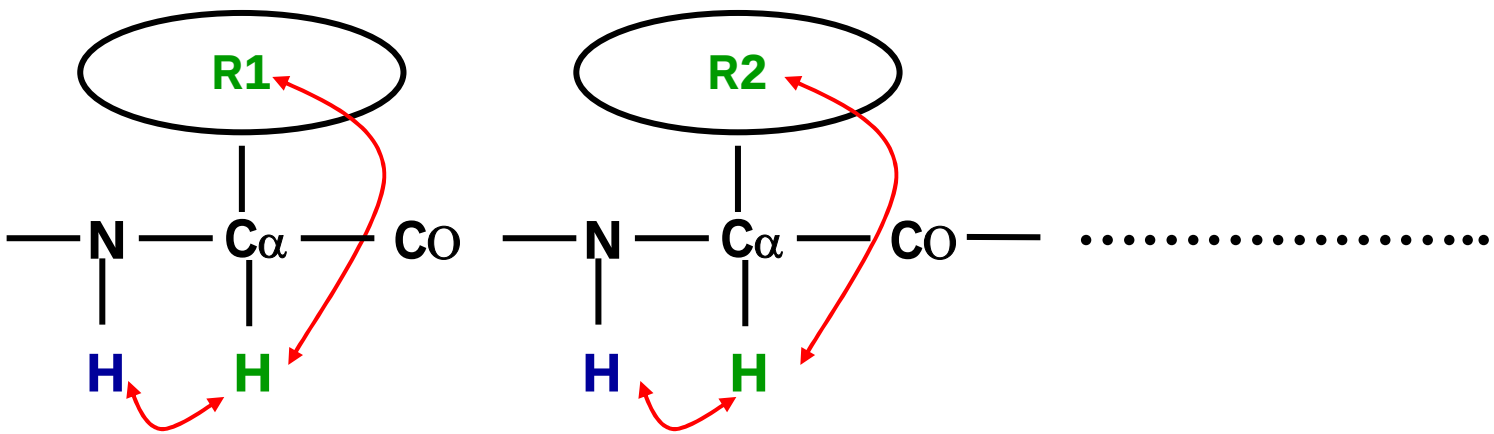


2D Homo Nuclear

1H-1H



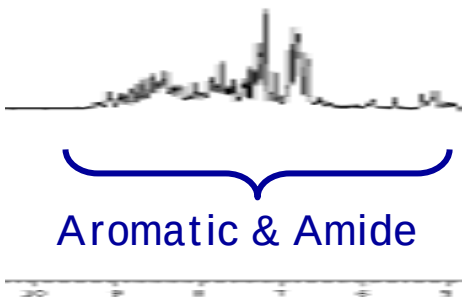


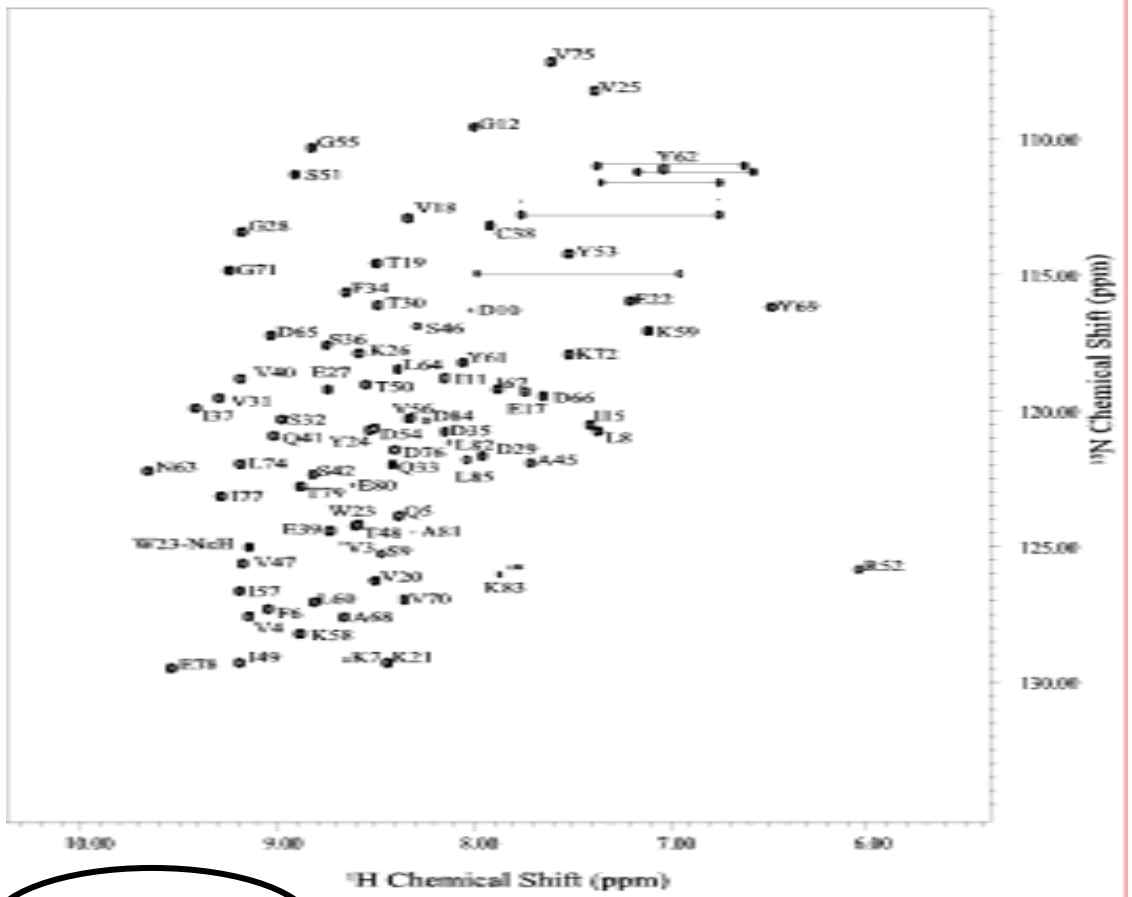


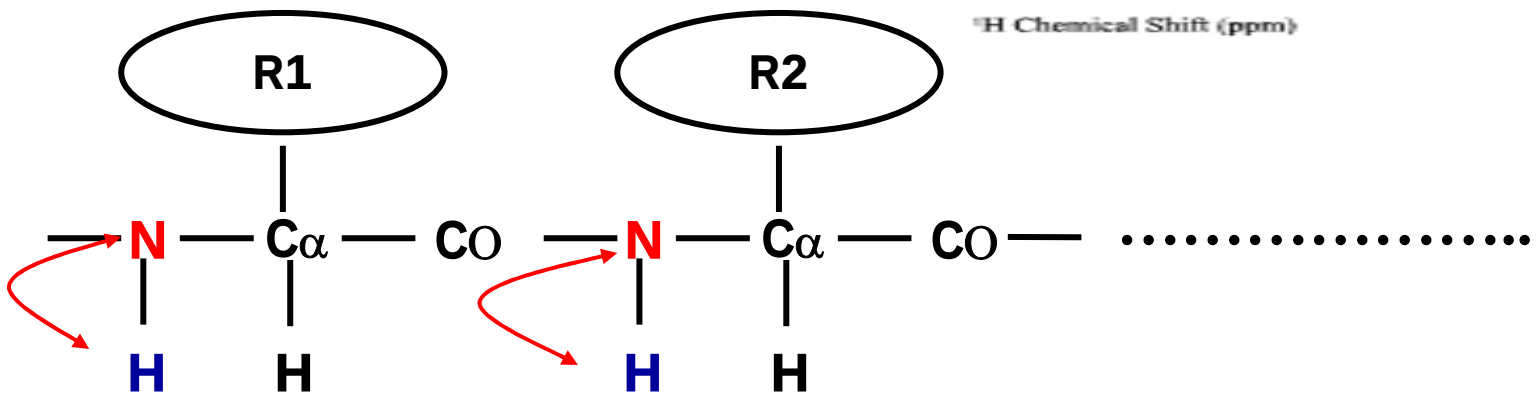
2D Hetero Nuclear

1H-15N

2D HSQC

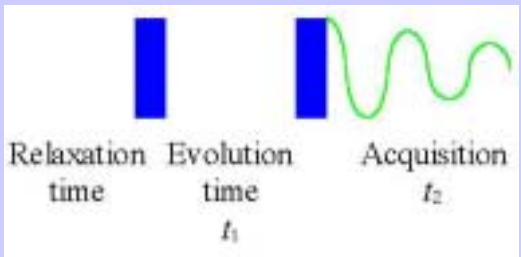






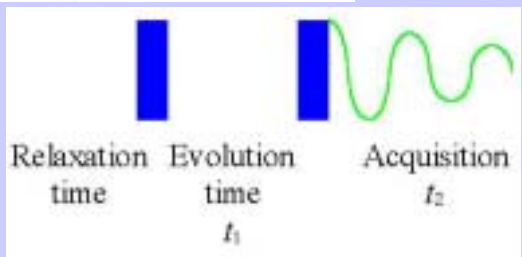
Multi-Dimensional NMR

Basic 3D Experiment

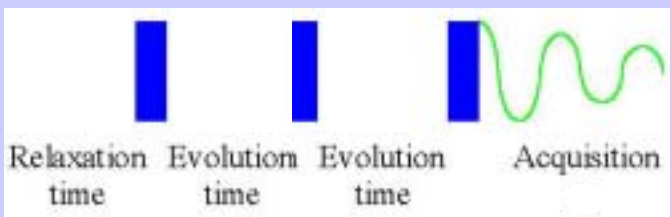


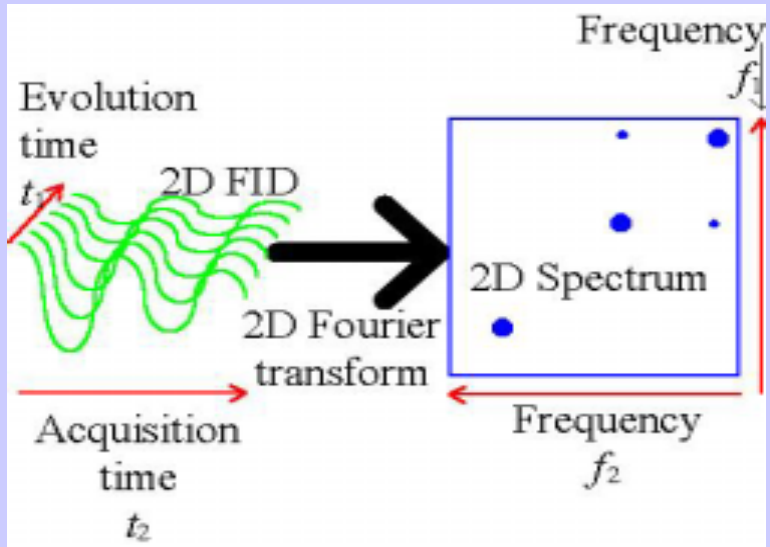
2D

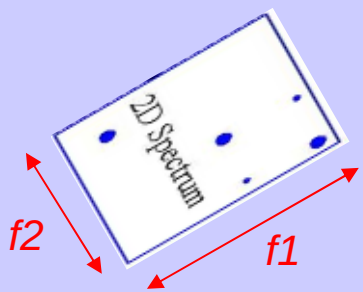
2D

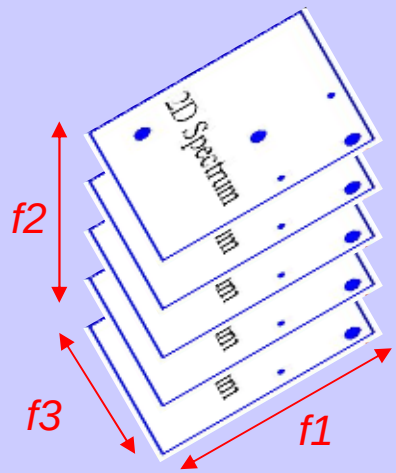


3D





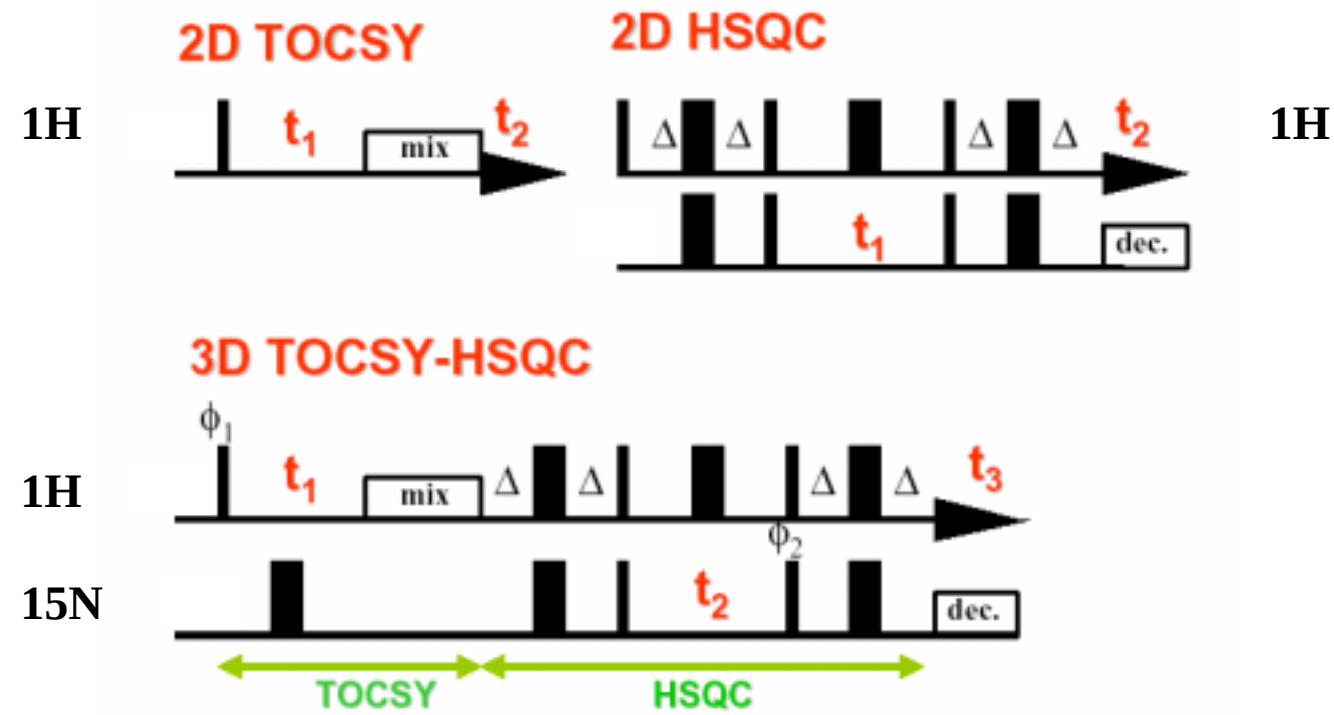


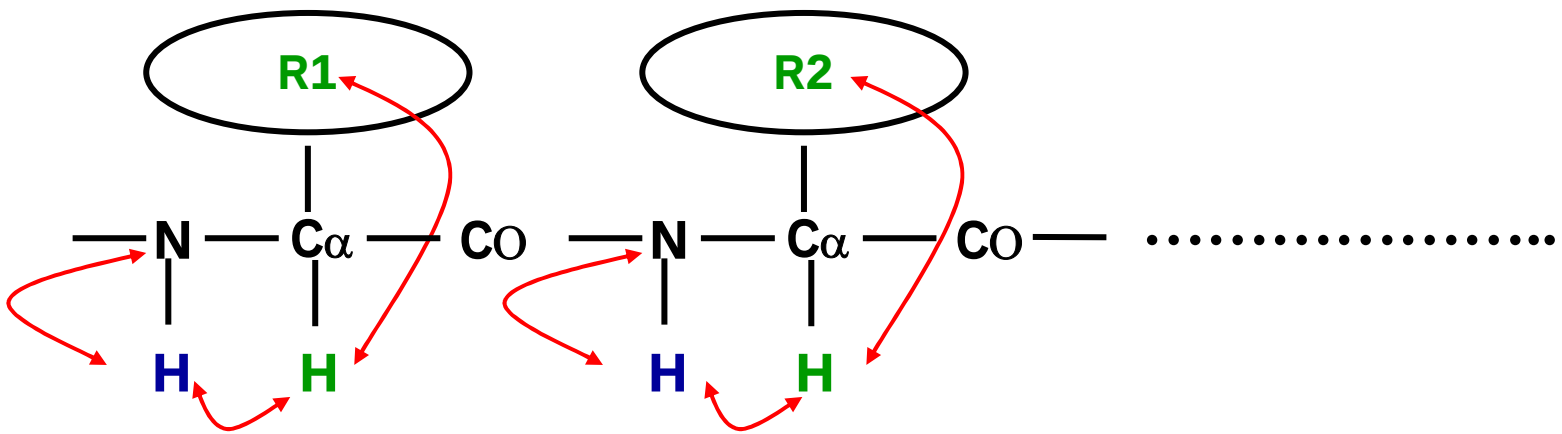


3D Hetero Nuclear

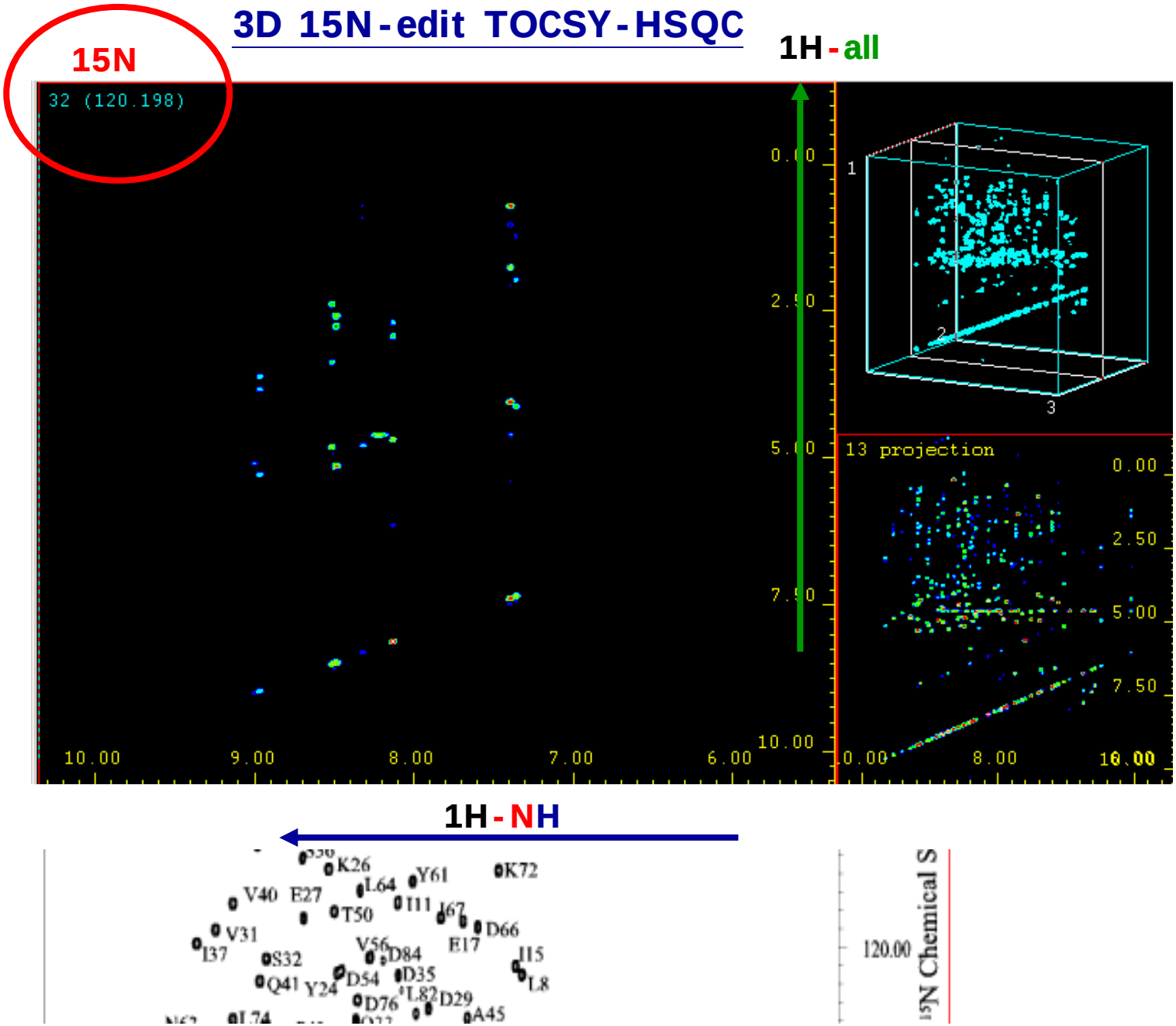
1H-15N-1H

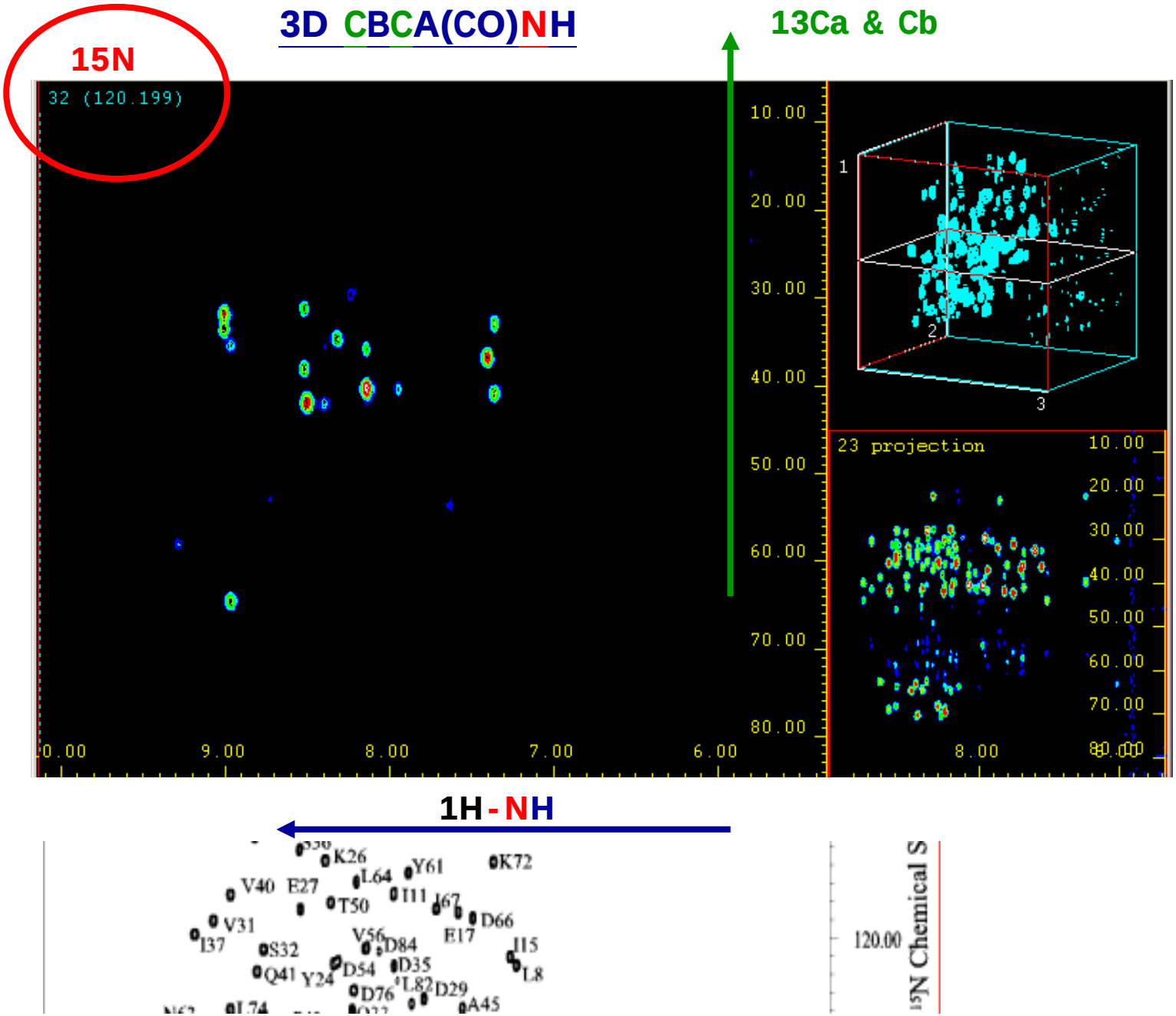
Fig. 1. The 3D pulse sequence.





3D 15N-edit TOCSY-HSQC




3D CBCA(CO)NH
13Ca & Cb
15N
32 (120.199)
10.00
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1
2
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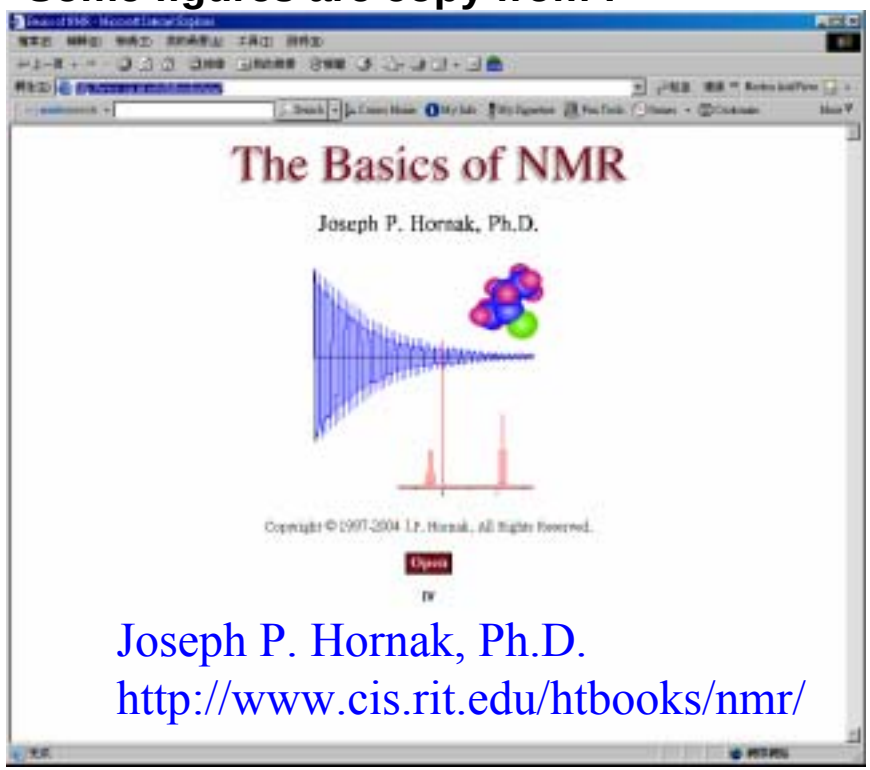
23 projection
1H-NH
K26
L64
Y61
K72
V40
E27
T50
I11
D66
V31
I37
S32
V56
D84
E17
I15
Q41
Y24
D54
D35
L8
D76
L82
D29
A45
120.00
Chemical S

Thank you!!

cfchang@ibms.sinica.edu.tw

Some figures are copy from :



Joseph P. Hornak, Ph.D.
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