2023 NMR Users Training (II)

Basic NMR SOP for
Small Molecules & Metabolomics Analysis

Basic NMR Concept & Applications

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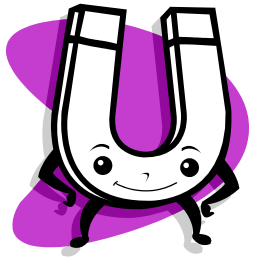
What is

Nuclear Magnetic Resonance?



What you should know about N, M, and R?

(Just point out few "terms" here)

Properties of the Nucleus (原子核的特性)

Nuclear spin & gyromagnetic ratio

Nuclear magnetic moments

NMR Active Nucleus in a Magnetic Field (磁場中的原子核)

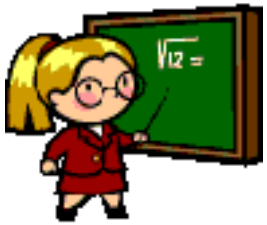
Precession and the Larmor frequency

Nuclear Zeeman effect & Boltzmann distribution

When the Spinning Nucleus receive the "Right" Energy

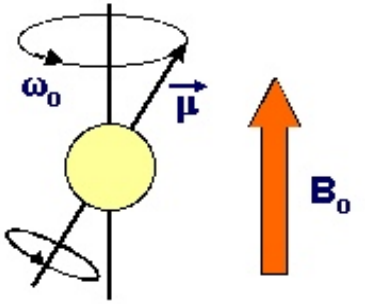
Nuclear Magnetic Resonance

(1) Properties of the Nucleus – Spin Parameters



μ (Magnetic Moment) = γ I ($h/2\pi$)

$6.626*10^{-34}$ joul-sec
(Plank Constant)



I: spin number (property of nucleus)

A (atomic mass) = Z(proton) + N(neutrons)

A	Z	N	Spin I
Even	Even	Even	I=0
Even	Odd	Odd	I=Integer 1,2,3....
Odd	Even	Odd	I=n/2, n=1,3,5...
Odd	Odd	Even	

γ : gyromagnetic ratio (property of nucleus)

Only those nucleus with spin I≠0 are NMR Active!!

JEOL
Solutions for Innovation

Table of Isotopes and NMR Parameters

- 1/2 spin
- half-integer spin
- integer spin
- not observable
- radioactive

Li 6 7 1 3/2 7.59 92.41 6.27 16.55 3.79 1590 15	Be 9 -3/2 100 -5.98 81.5 40						H 1 2 1/2 1 99.98 1.15E-02 42.58 6.54 5870 6.52E-03 10	H 3 1/2 -- 45.41 ☢						B 10 11 3 3/2 19.9 80.1 4.58 13.66 23.2 777 150	C 13 1/2 1.07 10.71 1 200	N 14 15 1 -1/2 99.63 0.36 3.08 -4.32 5.9 2.25E-02 1000	O 17 -5/2 3.8E-02 -5.77 6.50E-02 700	F 19 -1/2 100 40.08 4900 1200	Ne 21 3/2 0.27 -3.36 3.91E-02
Na 23 3/2 100 11.27 545 40	Mg 25 -5/2 10 -2.61 1.58 70												Al 27 5/2 100 11.1 1220 400	Si 29 -1/2 4.68 -8.47 2.16 400	P 31 1/2 100 17.25 391 1100	S 33 3/2 0.76 3.27 0.101 900	Cl 35 37 3/2 3/2 75.78 24.5 4.18 3.48 21 3.87 1100	Ar
K 39 41 3/2 3/2 93.25 6.73 1.99 1.09 2.79 3.33E-02 120	Ca 43 -7/2 0.13 -2.87 5.10E-02 70	Sc 45 7/2 100 10.36 1780 350	Ti 47 49 -5/2 -7/2 7.44 5.41 -2.40 -2.41 0.918 1.2 2400	V 50 51 6 7/2 0.25 99.75 4.25 11.21 0.818 2250 2500	Cr 53 -3/2 9.50 -2.41 0.507 2000	Mn 55 5/2 100 10.58 1050 3500	Fe 57 1/2 2.11 1.38 4.25E-03 12000	Co 59 7/2 100 10.08 1640 19000	Ni 61 -3/2 1.13 -3.81 0.24 1200	Cu 63 65 3/2 3/2 69.17 30.83 11.32 12.10 382 208 1000	Zn 67 5/2 4.10 2.67 0.692 450	Ga 69 71 3/2 3/2 60.1 39.89 10.25 13.02 246 335 1400	Ge 73 -9/2 7.73 -1.49 0.642 1100	As 75 3/2 100 7.32 149 700	Se 77 1/2 7.63 8.16 3.15 2500	Br 79 81 3/2 3/2 50.69 49.31 10.70 11.54 2.37 288 4500	Kr 83 9/2 11.49 -1.64 1.28
Rb 85 87 5/2 3/2 72.17 27.83 4.13 13.98 45 290 200	Sr 87 -9/2 7 -1.85 1.12	Y 89 -1/2 100 -2.09 0.7 500	Zr 91 -5/2 11.22 -3.97 6.26 1300	Nb 93 9/2 100 10.45 2870 3600	Mo 95 97 5/2 -5/2 15.92 9.55 -2.79 -2.85 3.06 1.95 5500	Tc 99 9/2 -- 9.62 ☢ 9000	Ru 99 101 -5/2 -5/2 12.76 17.06 -1.96 -2.19 0.848 1.59 9000	Rh 103 -1/2 100 -1.35 0.186 12000	Pd 105 -5/2 22.33 -1.96 1.49	Ag 107 109 -1/2 -1/2 51.83 48.16 -1.73 -1.99 0.205 0.29 850	Cd 111 113 -1/2 -1/2 12.8 12.22 -9.07 -9.49 7.27 7.94 950	In 113 115 9/2 9/2 4.29 95.71 9.37 9.39 88.5 1980 1100	Sn 117 119 -1/2 -1/2 7.68 8.59 -15.26 -15.97 20.8 26.6 600	Sb 121 123 5/2 7/2 57.21 42.79 10.26 5.55 548 117 1300	Te 123 125 -1/2 -1/2 0.89 7.07 -11.23 -13.55 0.961 13.4 4500	I 127 5/2 100 8.58 560 4000	Xe 129 131 1/2 3/2 26.44 21.18 -11.86 3.52 33.6 3.5 7000
Cs 133 7/2 100 5.62 284 500	Ba 135 137 3/2 3/2 6.59 11.23 4.26 4.76 1.93 4.62	La 138 139 5 7/2 9.00E-02 99.91 5.66 6.06 0.497 356 1200	Hf 177 179 7/2 -9/2 18.6 13.62 1.73 -1.09 1.54 0.438	Ta 181 7/2 99.98 5.16 220	W 183 1/2 14.31 1.8 6.31E-02 7000	Re 185 187 5/2 5/2 37.4 62.6 9.72 9.82 305 526	Os 187 189 1/2 3/2 0.99 3.35 1.43E-03 2.32 5500	Ir 191 193 3/2 3/2 37.3 62.6 0.77 0.83 6.38E-02 0.137	Pt 195 1/2 33.83 9.29 20.7 15000	Au 197 3/2 100 0.75 0.162	Hg 199 201 1/2 -3/2 16.87 13.18 7.71 -2.85 5.89 1.16 3500	Tl 203 205 1/2 1/2 29.52 70.47 24.73 24.97 340 836 6500	Pb 207 1/2 22.1 8.88 11.8 18000	Bi 209 9/2 100 6.96 848	Po 209 1/2 ☢	At 210 211 5 -9/2 ☢	Rn 219 5/2 ☢
Ce	Pr 141 5/2 100 13.04 0	Nd 143 145 -7/2 -7/2 12.2 8.3 -2.32 -1.430 0	Pm 147 7/2 ☢	Sm 147 149 -7/2 -7/2 14.99 13.82 -1.78 -1.46 0 0	Eu 151 153 5/2 5/2 47.81 52.19 10.59 4.67 0 0	Gd 155 157 -3/2 -3/2 14.8 15.65 -1.31 -1.71 0 0	Tb 159 3/2 100 10.24	Dy 161 163 5/2 5/2 18.91 24.9 -1.46 2.05 0 0	Ho 165 7/2 100 9.09 0	Er 167 -7/2 22.93 -1.23 0	Tm 169 -1/2 100 -3.53 0	Yb 171 173 1/2 -5/2 14.28 16.13 7.53 -2.07 0 0 1300	Lu 175 176 7/2 7 97.41 2.59 4.86 3.45 0 0				
		U 235 7/2 0.72 -0.83 ☢															

The rest of the actinide series is not commonly observed due to their radioactive nature.

Element

	C
· Isotope	13
· Spin	1/2
· Natural Abundance	1.07
· Gamma (g/10^6 Hz/T)	10.71
· Relative Receptivity to 13C	1
· Typical Chemical Shift Range (ppm)	200

JEOL ECZ NMR Frequencies

Nuc.	9.4T	11.7T	14.1T	Nuc.	9.4T	11.7T	14.1T
1H	399.78	500.16	600.17	105Pd	18.29	22.89	27.46
3H	61.37	76.78	92.13	107Ag	16.18	20.25	24.29
3He	304.56	381.03	457.22	111Cd	84.76	106.04	127.24
6Li	58.83	73.60	88.32	113Cd	88.67	110.93	133.11
7Li	155.37	194.38	233.25	113In	87.42	109.36	131.23
9Be	56.16	70.28	84.33	115In	87.60	109.59	131.51
10B	42.95	53.74	64.48	115Sn	142.45	178.22	213.85
11B	128.27	160.47	192.56	119Sn	149.68	186.31	223.81
13C	100.53	125.77	150.91	121Sb	95.47	119.39	143.62
14N	28.89	36.14	43.37	123Sb	51.81	64.82	77.78
15N	40.51	50.68	60.82	123Te	104.62	130.89	157.05
17O	54.20	67.80	81.36	125Te	126.13	157.80	189.35
19F	376.17	470.62	564.73	127I	79.99	100.07	120.09
21Ne	31.56	39.51	47.41	129Xe	110.87	138.73	166.50
23Na	105.75	132.30	158.76	131Xe	33.61	41.30	49.52
25Mg	24.47	30.62	36.74	133Cs	52.44	65.60	78.72
27Al	104.17	130.33	156.39	135Ba	39.72	49.69	59.62
29Si	79.43	99.37	119.24	137Ba	44.43	55.58	66.70
31P	161.83	202.47	242.86	138La	53.16	66.51	79.81
33S	30.69	38.39	46.07	139La	56.47	70.65	84.78
35Cl	39.17	49.01	58.80	141Pr	122.40	153.13	183.75
37Cl	32.61	40.79	48.95	143Nd	21.77	27.24	32.69
39K	18.65	23.34	28.01	145Nd	13.42	16.79	20.14
41K	10.24	12.81	15.37	147Sm	16.66	20.85	25.02
43Ca	26.91	33.66	40.39	149Sm	13.74	17.19	20.62
45Sc	97.11	121.50	145.79	151Eu	98.39	124.35	149.22
47Ti	22.54	28.20	33.83	153Eu	43.69	54.91	65.89
49Ti	22.54	28.20	33.84	155Gd	12.37	15.47	18.55
50V	39.86	49.87	59.84	157Gd	16.22	20.29	24.34
51V	105.15	131.56	157.86	159Tb	96.06	120.18	144.21
50Cr	22.60	28.27	33.92	161Dy	13.76	17.21	20.65
53Mn	99.10	123.99	148.78	163Dy	19.26	24.09	28.91
57Fe	12.94	16.19	19.43	165Ho	85.54	106.76	128.13
59Co	94.86	118.67	142.40	167Er	11.53	14.43	17.31
61Ni	35.72	44.69	53.63	169Tm	33.16	41.48	49.77
63Cu	106.05	132.68	159.21	171Yb	70.67	88.41	106.09
65Cu	113.61	142.13	170.55	173Yb	19.46	24.35	29.22
67Zn	25.01	31.29	37.56	175Lu	45.66	57.12	68.54
69Ga	95.95	120.05	144.06	176Lu	32.40	40.54	48.65
71Ga	121.92	152.53	183.00	177Hf	16.23	20.30	24.35
73Ge	13.95	17.45	20.94	179Hf	10.19	12.75	15.30
75As	68.45	85.64	102.77	181Ta	47.93	59.97	71.99
77Se	76.24	95.39	114.46	183W	16.66	20.84	25.01
79Br	100.15	125.31	150.37	185Re	95.65	119.66	143.59
81Br	107.97	135.08	162.09	187Re	96.96	113.79	136.55
83Kr	15.44	19.32	23.18	187Os	9.12	11.42	13.70
85Rb	38.60	48.29	57.95	189Os	31.64	38.84	46.61
87Rb	130.91	163.66	196.38	191Ir	7.19	9.00	10.80
87Sr	17.33	21.68	26.01	193Ir	7.81	9.77	11.73
89Y	19.59	24.51	29.41	195Pt	85.84	107.32	128.02
91Zr	37.16	46.50	55.79	197Au	6.95	8.70	10.44
93Nb	97.85	122.42	146.90	199Hg	71.60	89.58	107.50
95Mo	26.05	32.59	39.11	201Hg	26.73	33.44	40.13
97Mo	26.69	33.38	39.93	203Tl	208.77	261.18	313.44
99Tc	90.42	113.13	135.75	205Tl	231.62	289.82	347.82
99Ru	18.45	23.08	27.69	207Pb	83.39	104.32	125.18
101Ru	20.67	25.86	31.04	209Bi	64.24	80.37	96.44
103Rh	12.74	15.94	19.12	235U	7.79	9.75	11.70

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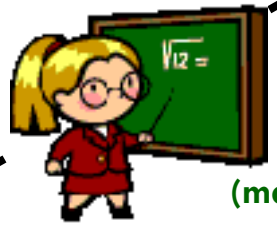
(2-1) NMR Active Nucleus in Magnetic Field – Larmor precession

有核磁共振特性的原子核, 在磁場中會像陀螺一樣自旋並產生磁矩(magnetic moment) , 此自旋頻率稱之為"Larmor frequency "

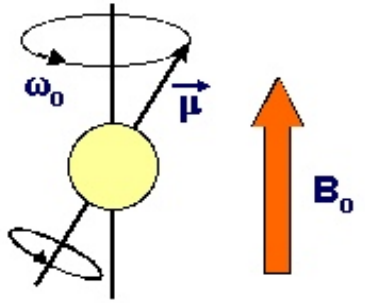


$$\mu = \gamma I (h/2\pi)$$

(magnet moment)

γ (gyromagnetic ratio)

$6.626*10^{-34}$ joul-sec (Plank Constant)



$$\upsilon_{0,precession} = (\gamma B_0/2\pi)$$

or $\omega_0 = \gamma B_0$

- Under the same magnetic field, precession frequency proportional to the gyromagnetic ratio γ of each atom (自旋頻率與原子核的特性有關)

  $\gamma_{(1H)}$=26.7519 (10^7*rad/T*s) $\gamma_{(13C)}$=6.7624 (10^7*rad/T*s)

- The higher the magnetic field, faster the spinning frequency (磁場愈高轉速愈快)

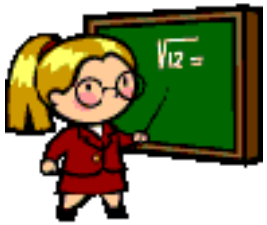
Differ "atom type" ➔ different Larmor frequency

核種不同 (ie.磁旋比 gyromagnetic ratio γ 不同), 自旋的頻率就不同!

14.1 Tesla

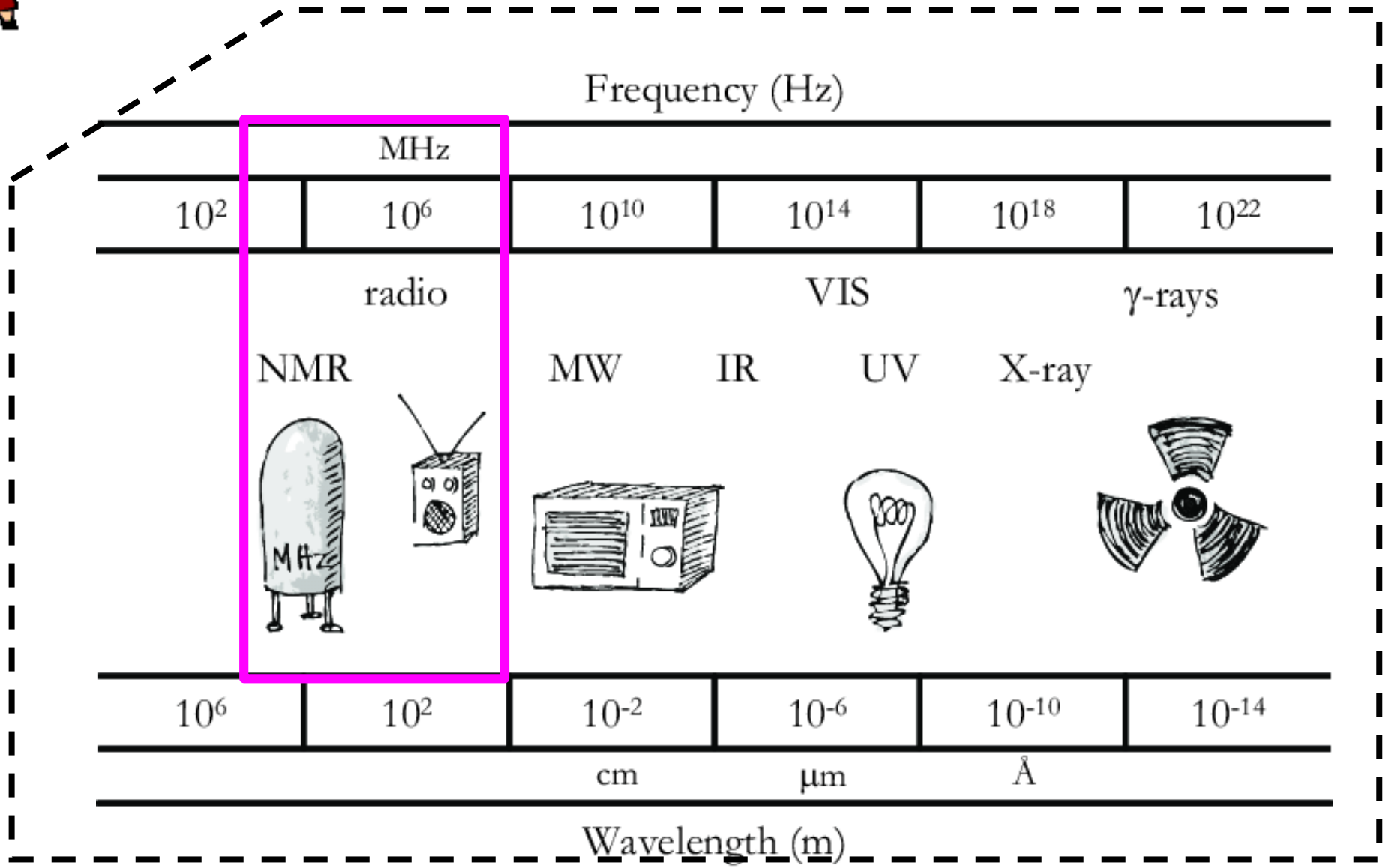
1H γ: 26.7519 (10^7*rad/T*s)
13C γ : 6.7262 (10^7*rad/T*s)
15N γ : -2.7116 (10^7*rad/T*s)

$$\upsilon_{0,precession} = (\gamma B_0/2\pi)$$



The precession frequency is @ radio frequency range

自旋頻率接近無線電頻率 (radio frequency)



Same "atom type", but "under different chemical environment " could generate different signal. This makes NMR useful and interesting.

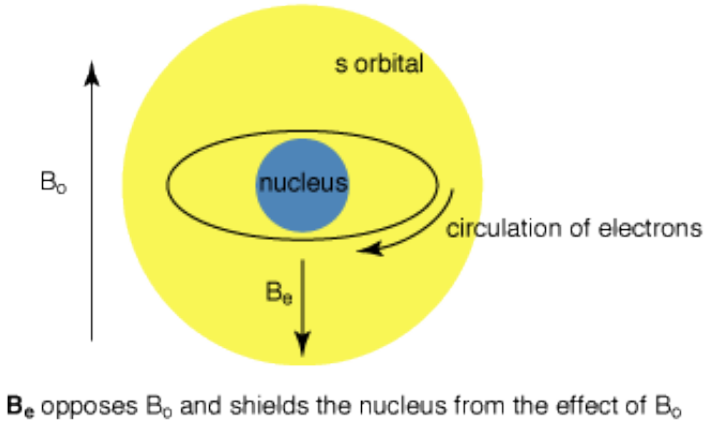
$$\upsilon_{0,precession} = (\gamma B_0/2\pi) \rightarrow \upsilon_{precession} = (\gamma B_{eff}/2\pi)$$

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

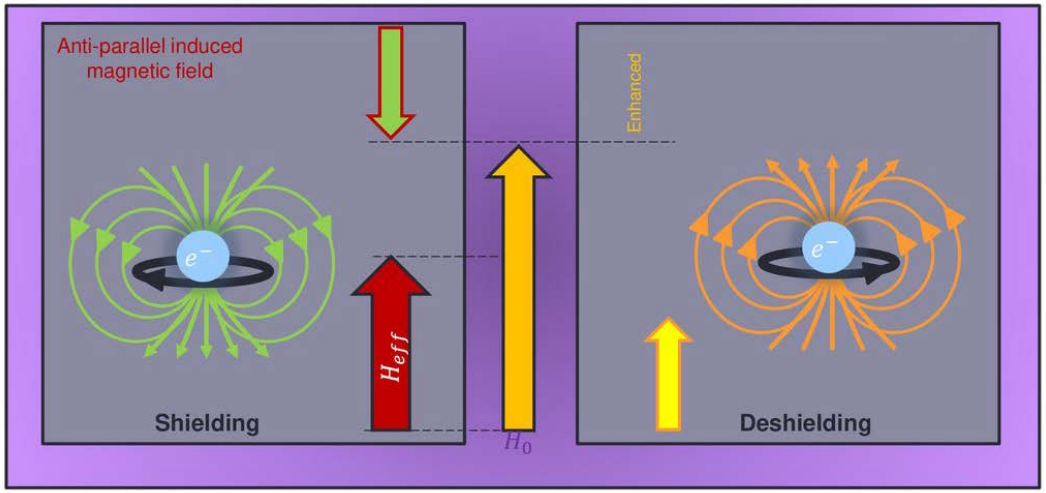
$$\upsilon_{precession} = \upsilon_{0,\ precession}\ (1-\sigma)$$

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

$\sigma < 0$ → electron around this nuclei is withdraw



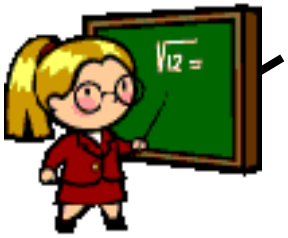
B_e opposes B_o and shields the nucleus from the effect of B_o



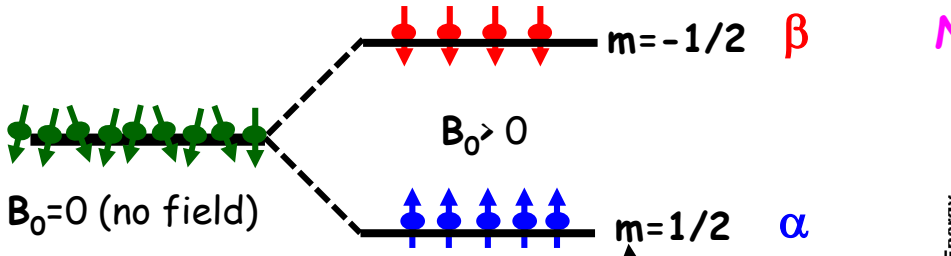
Published by Matthew Wade

(2-2) NMR Active Nucleus in a Magnetic Field – Zeeman Effect

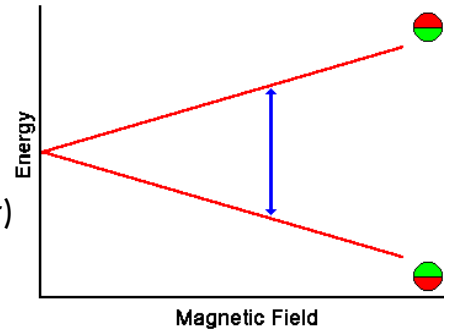
在磁場中, 核能階分裂(Nuclear Zeeman effect), 且核自旋以Boltzmann distribution 分布



Example: I=1/2 system, spins tend to generally aligned with or opposed to the external field, as " spin-up" or "parallel", and "spin-down" or "anti-parallel".



Nuclear Zeeman effect

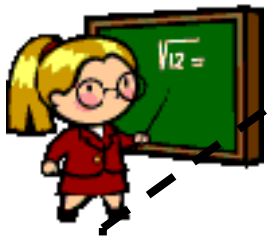


$N_\beta/N_\alpha = \exp(-\Delta E/kT) = \exp[(\gamma h B_o)/(2\pi kT)]$

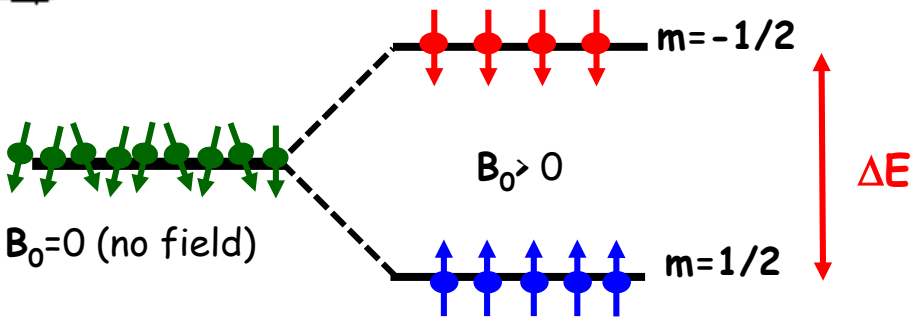
Boltzmann distribution

Different between Nβ and Nα just "ppm", ie. NMR is an "insensitive" technique

(3) When the Nucleus receive the "Right" Energy => Resonance



$\Delta E = rhB_0/2\pi = h\upsilon_{precession}$

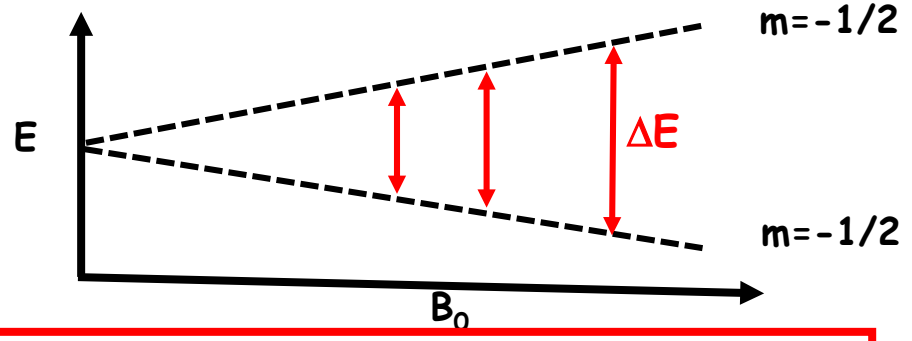


$E_{induced} = \Delta E$

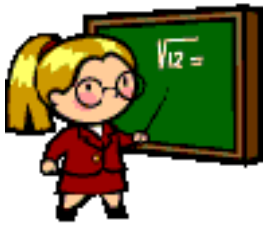
On Resonance !!!

(ex: $E_{induced} = h\upsilon_{induced}$)

The spin at low energy level absorb the Induce Energy, then jump to the higher energy level (m=1/2→m= - 1/2), but still spin at $\upsilon_{precession}$



Higher the field (B_0), better the sensitivity and resolution



Radiation energy for NMR spectroscopy

(頻率範圍約 80-1000MHz, 是能量弱的電磁波)

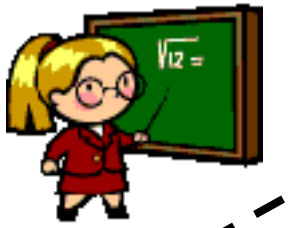
- Energy=hv ; C= λ v

λ: 1 | 10 | 10^2 | 10^3 | 10^4 | 10^5 | 10^6 | 10^7 | 10^8 | 10^9 nm

(the wave)	X-ray	UV/VIS	Infrared	Microwave	Radio Freq.
(the transition)	Electronic Transition		Vibration	Rotation	Nuclear Spin
(spectrometer)	X-ray	UV/VIS/CD	Infrared/Raman	Fluorescence	NMR

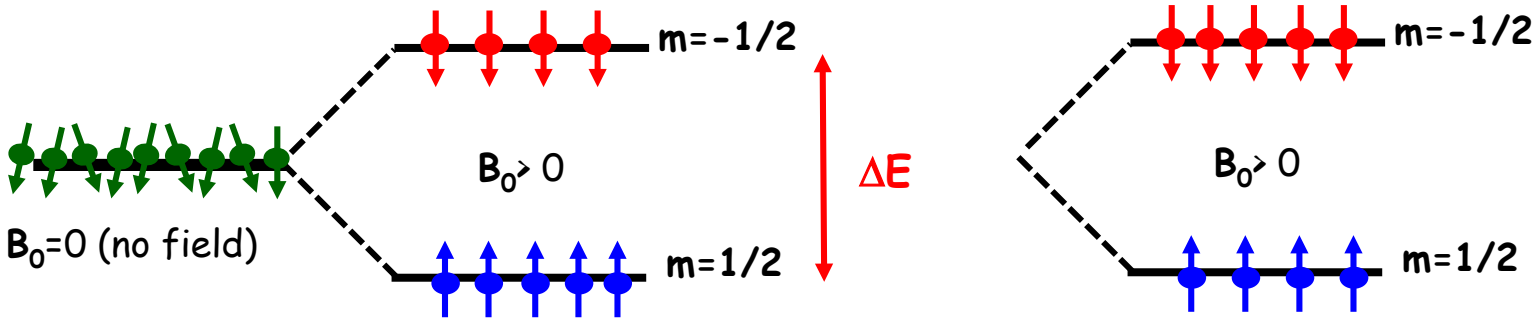
ν: 10^{11} | 10^{10} | 10^9 | 10^8 | 10^7 | 10^6 | 10^5 | 10^4 | 10^3 | 10^2 MHz

Spectroscopy Energy



During irradiation (current induced)

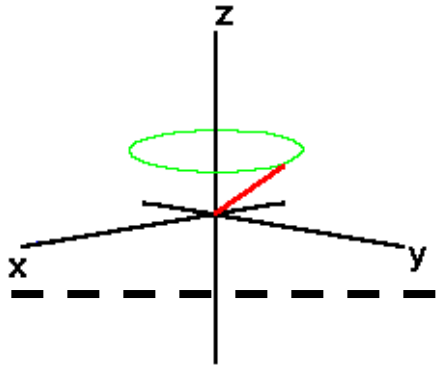
(1) Some Nuclei in lower energy sate will absorb induce energy , and jump to the higher energy state (ie 能階躍遷)

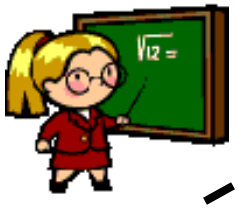


(2) All of the individual nuclear magnetic moments become phase coherent, and the net M process around the z axis at a angel α

$M_z = M\cos\alpha$

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



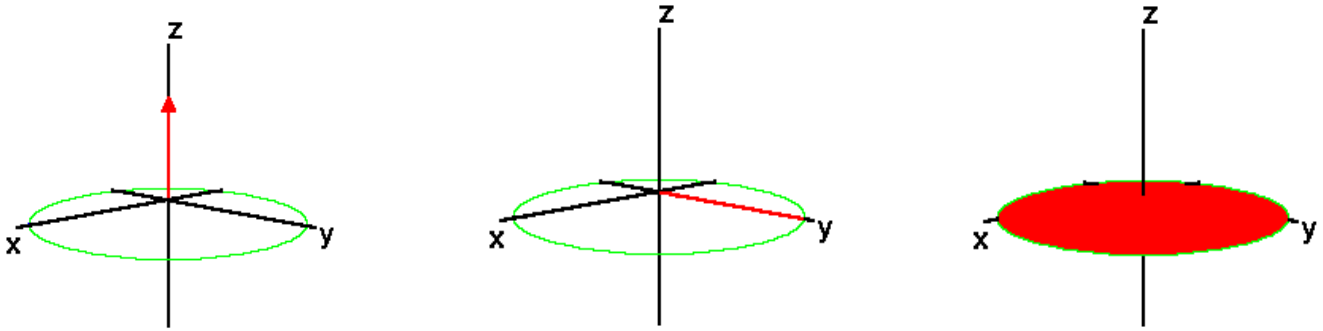


After irradiation ceases

(1) population of the states revert to a Boltzmann distribution

(2) individual nuclear magnetic moments begin to lose their phase coherence and return to a random arrangement around the z axis.

- This process is called "relaxation process"
- There are two types of relaxation process :
  - T1(spin-lattice relaxation) (ie. 回到最低能量)
  - T2(spin-spin relaxation) (ie. 回到最大亂度)



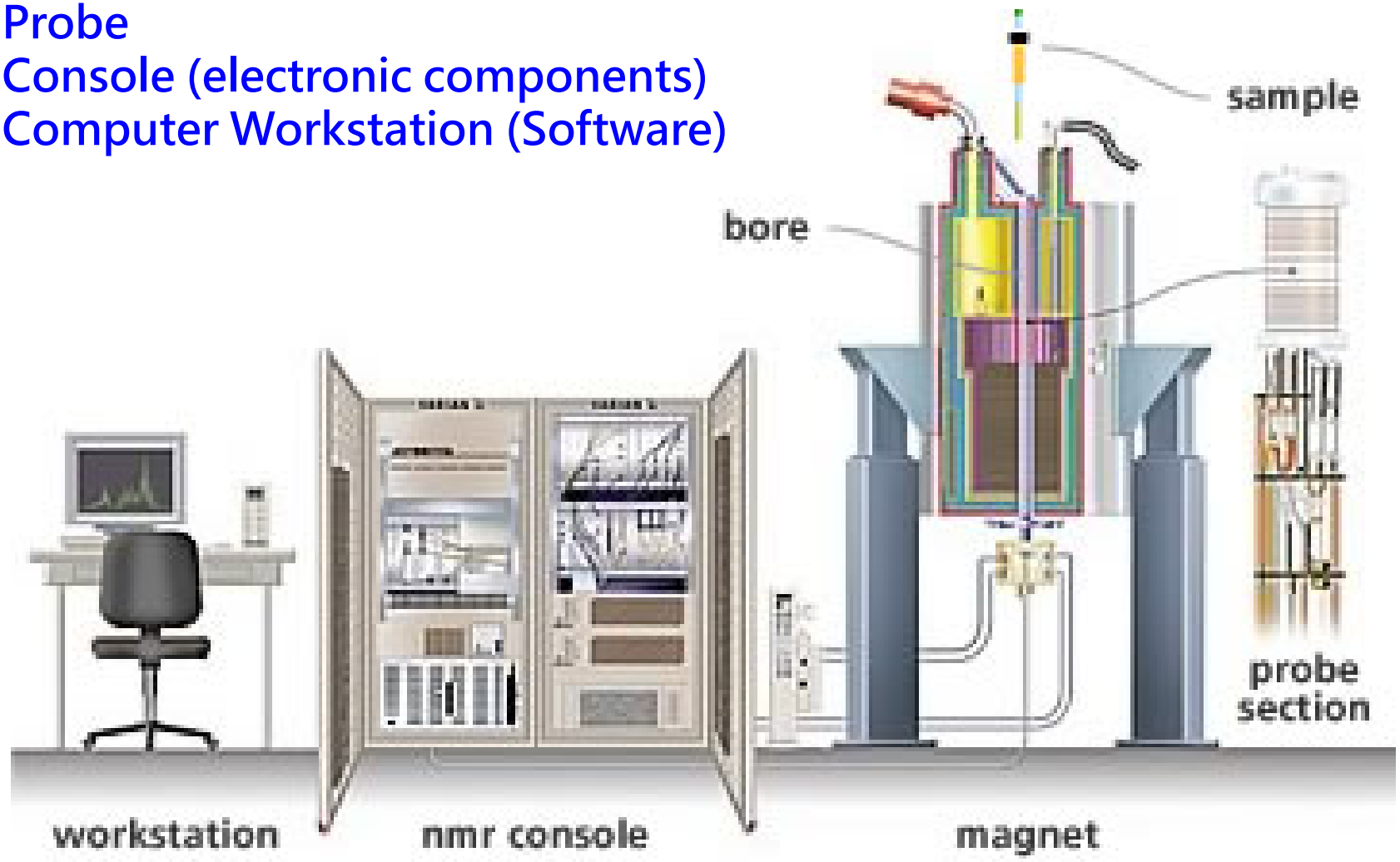
NMR signals will be collected during this relaxation process

How to

Obtain NMR Signals (Spectrum)?

NMR Hardware (核磁共振儀示意圖)

(1) Magnet (superconducting magnet)
(2) Probe
(3) Console (electronic components)
(4) Computer Workstation (Software)



Steps for NMR Experiments

Prepare Samples → Collect NMR spectra → Data Analysis

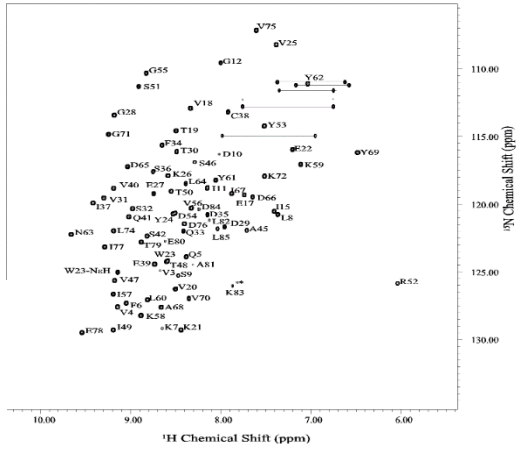
Expt. Methods

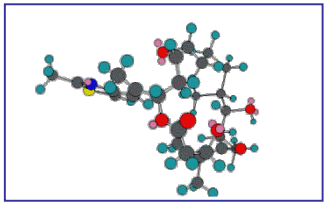
Expt, Design

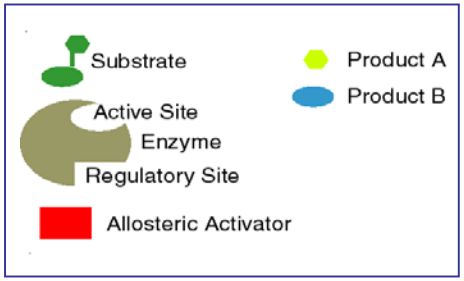
Processing / Analysis

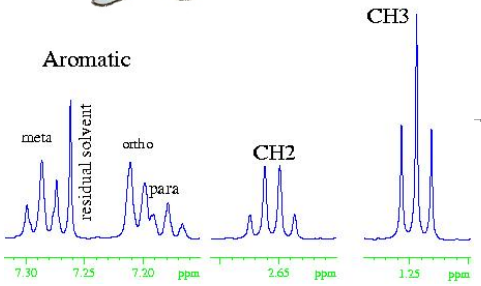
Assignment, Structure Calculation, Interpretation

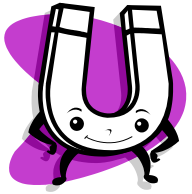
……..









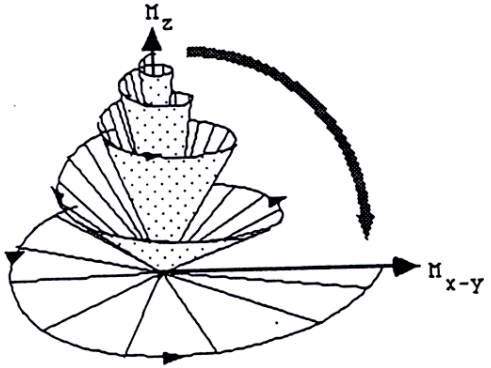


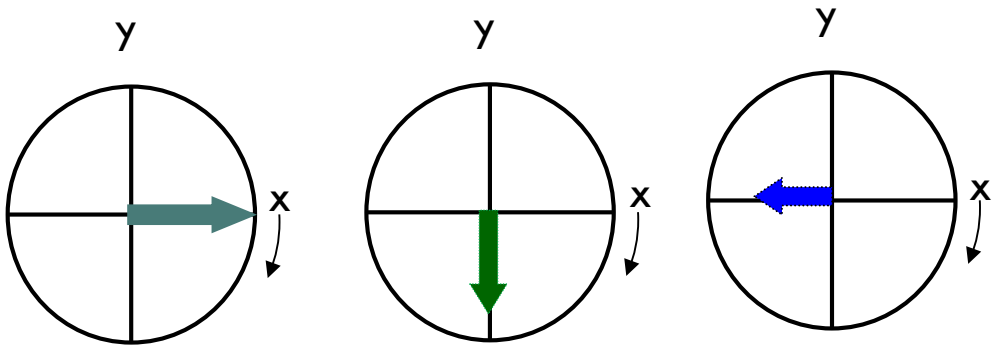
(1) NMR Signal -FID

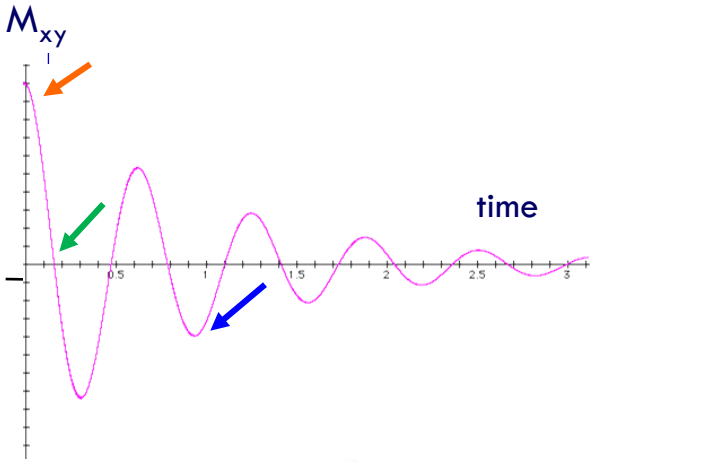
- FID is the time-domain response of the spin after a pulse (irradiation energy)

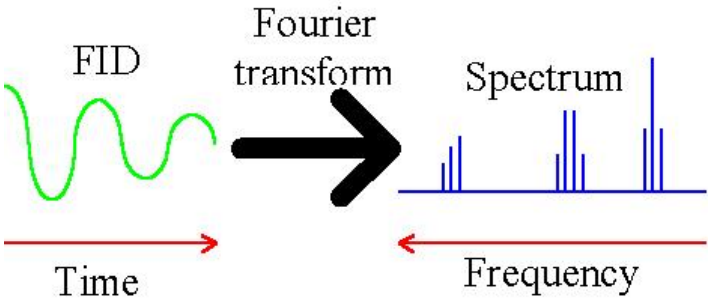
- Due to the relaxation effect, the response at receiver coil become a exponentially decaying signal, ie Free Induction Decay (FID).

Relaxation process

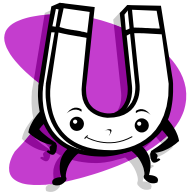








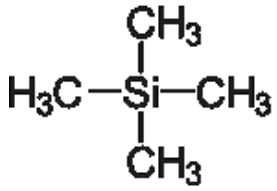
- The FID (free induction decay) Fourier transform to frequency domain to obtain $\upsilon_{precession}$ for each different nuclei.

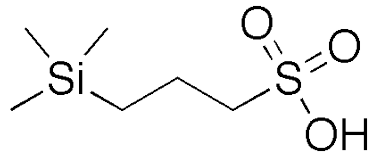


(2) NMR Signal –Chemical Shift

•The frequency of absorption for a nucleus of interest (υ)relative to the frequency of absorption of a molecular standard ($\upsilon_{standard}$) is called the chemical shift of the nucleus (notice the frequency υ is in unit of Hz).

•The standard molecule for both ^{1}H and ^{13}C NMR spectroscopy is tetramethylsilane (TMS). For biomolecular NMR, 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) is used instead.



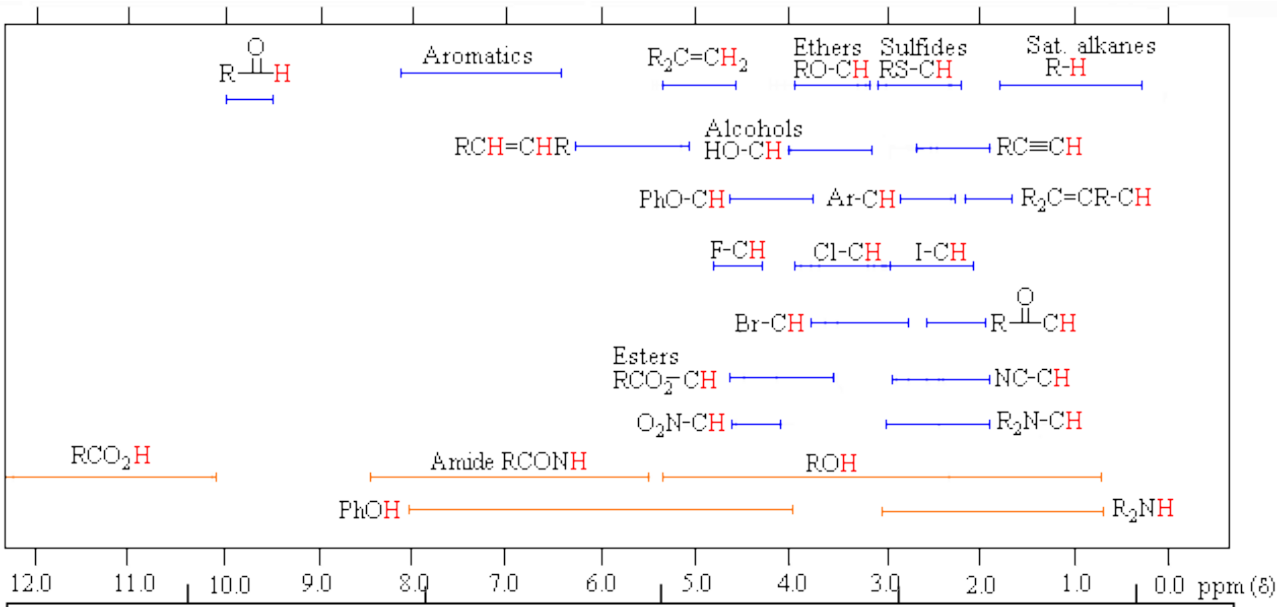


•Chemical shift is used to express as δ , and $\delta=(\upsilon-\upsilon_{TMS})/\upsilon_0$ where υ_0 is the center frequency for magnet (ex: 600MHz for 1H , 150MHz for 13C @14.1 T NMR)

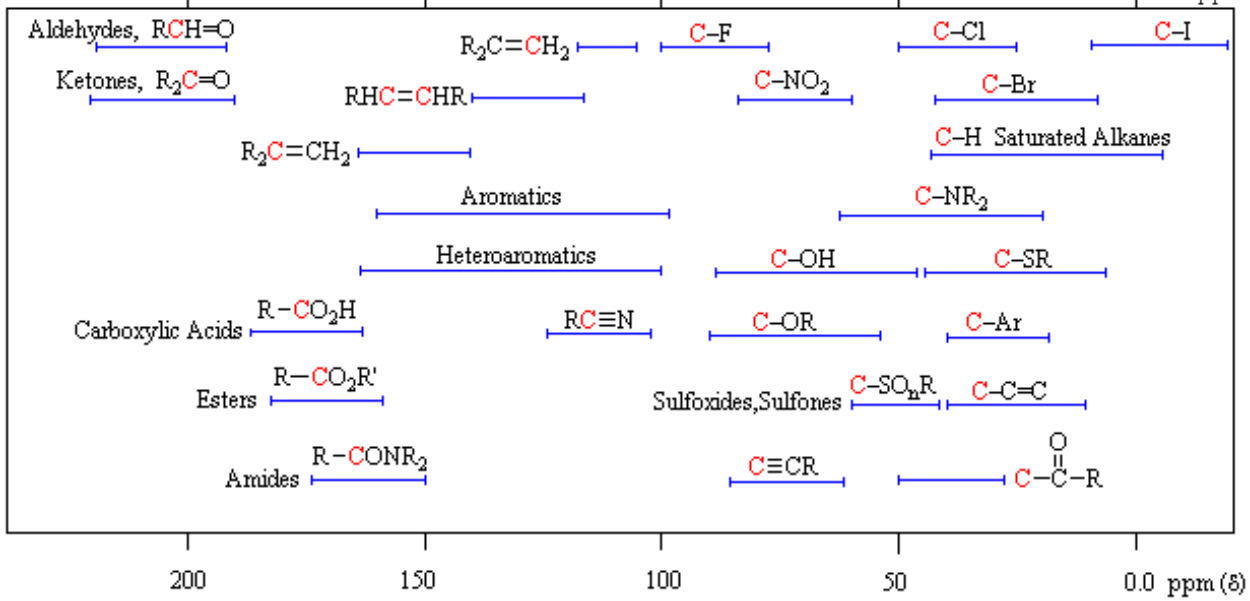
•Chemical shift δ is in the ratio of Hz/MHz, the result is a ratio of one part per million, ppm (which is independent of external magnetic field)

• Notice that, the chemical shifts for a molecule do not depend on the magnetic field. For example, chemical shift for H2O in 100 MHz magnet (2.35 T) is the same as in a 600 MHz magnet (14.1 T).

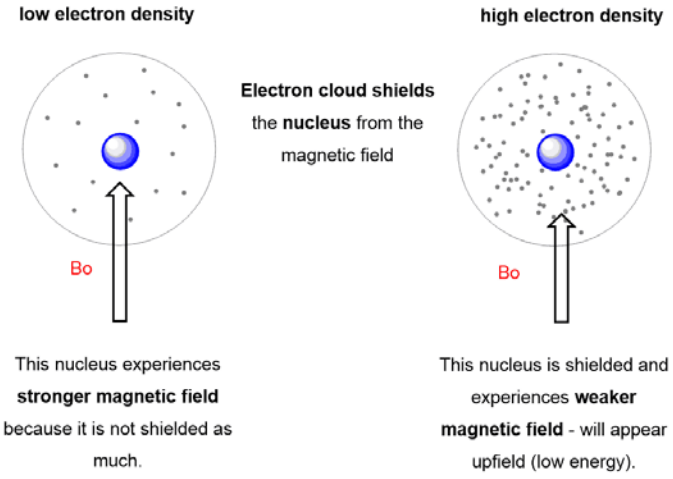
1H



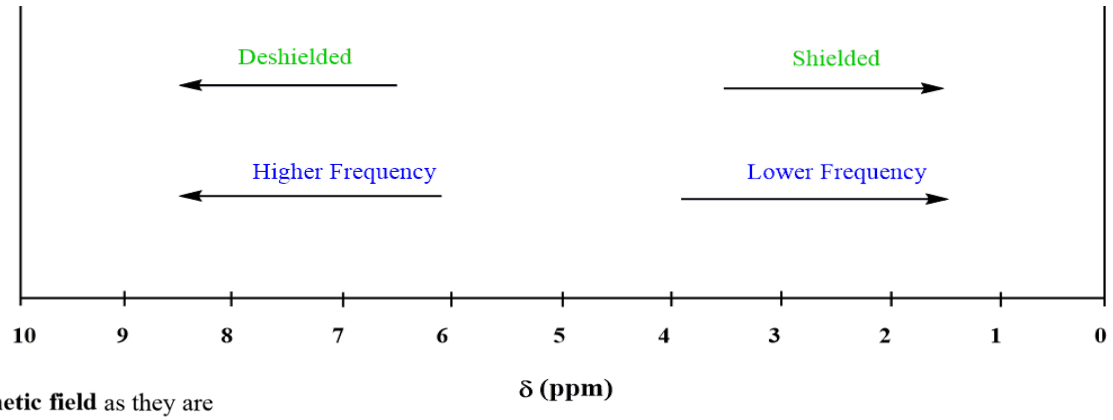
13C



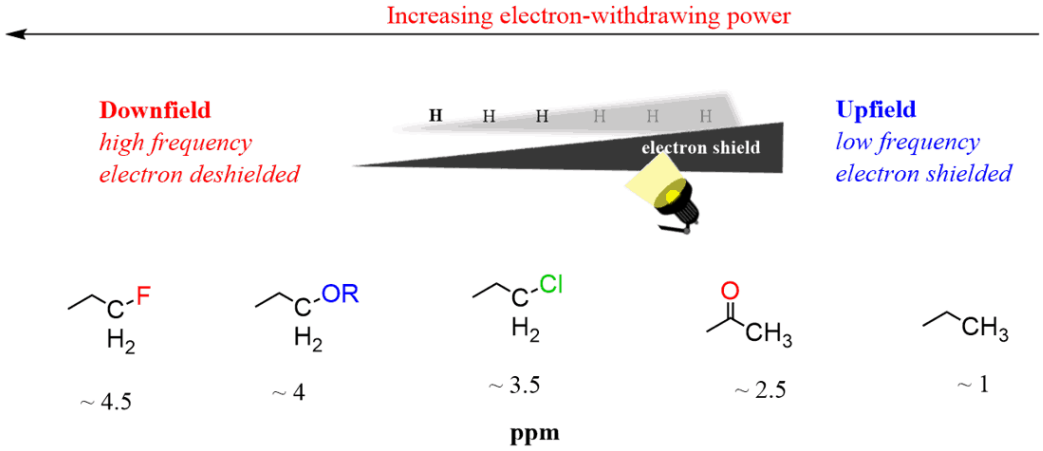
Electron Cloud



Scale of NMR Spectrum



The more electron-withdrawing the group, the stronger the protons feel the magnetic field as they are less shielded by the electrons and more exposed to the field.



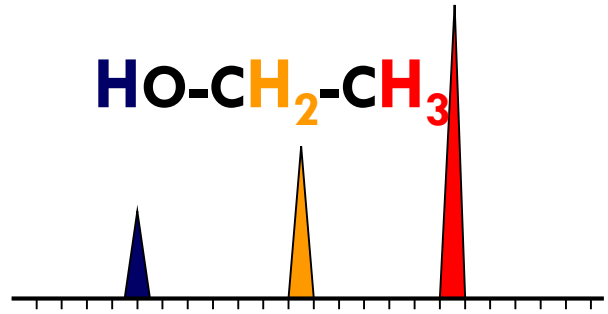
Fluorine, as the **most electronegative element, deshields and shifts** the signal of adjacent protons to ~ 4 ppm.

The **oxygen and chlorine are not as electronegative** and the ppm value is less downfield. I, Br, and some other groups also shift the signal to this region.

The **carbonyl group is electron-withdrawing** and the frequency of H's next to are shifted downfield by ~ 1.5 and found at 2.5 ppm.

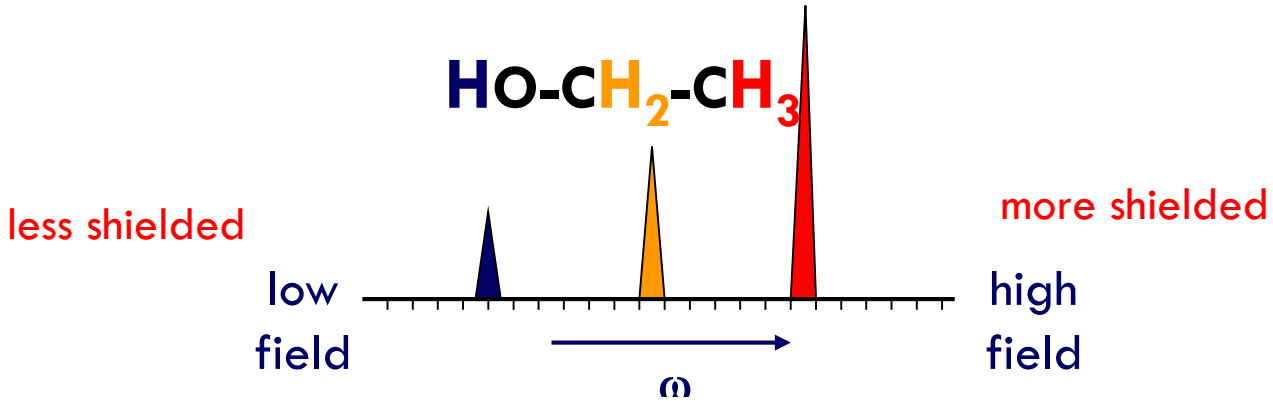
This is **the typical region of alkyl protons**. Increasing the number of alkyl groups shifts the signal downfield.

Ethanol

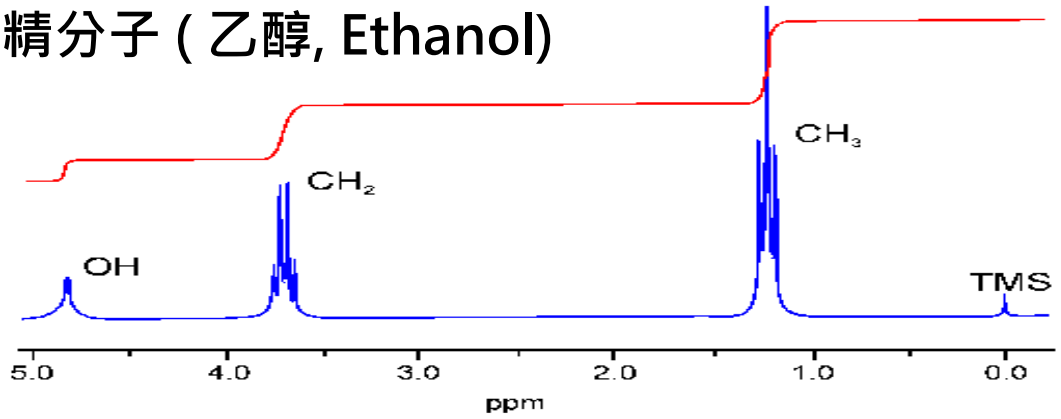


What a simple 1D 1H NMR spectrum tell us?

- The *number* of signals shows how many different kinds of protons
- The *location* of the signals shows how shielded/deshielded the proton
- The *intensity* of the signal shows the number of protons of that type.
- Signal *splitting* shows the number of protons on adjacent atoms.

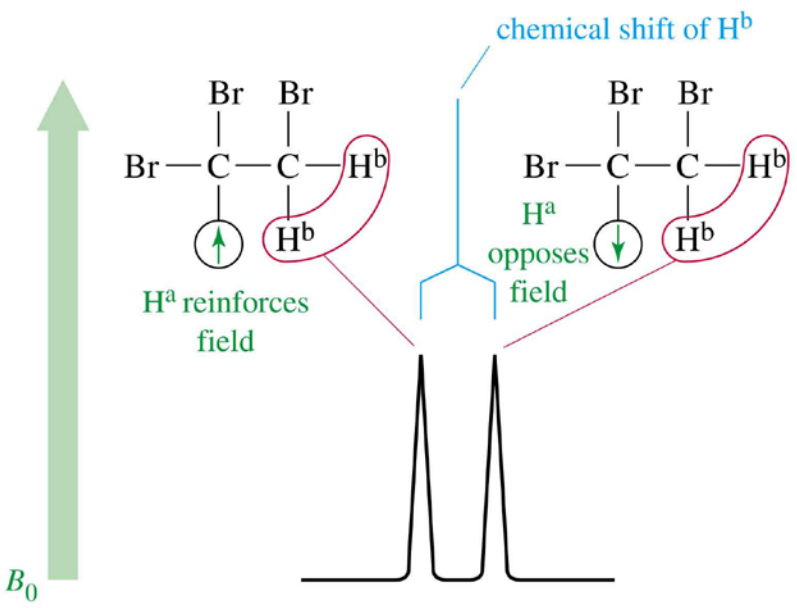


Example: 酒精分子 (乙醇, Ethanol)

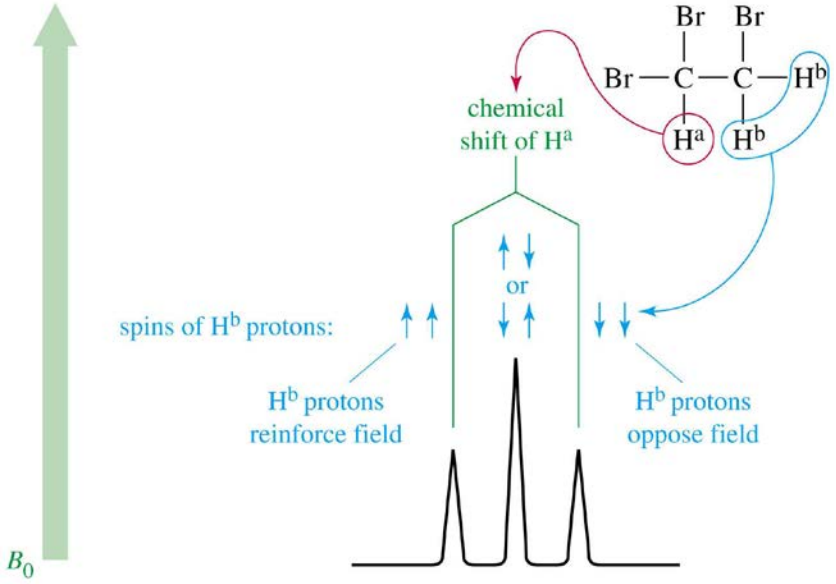


(3) NMR Signal –Coupling Effect

- 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*.



Chemical Shift of H^b

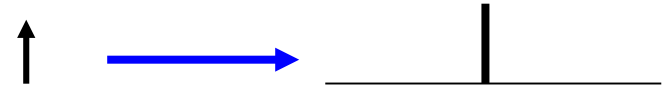


Chemical Shift of H^a

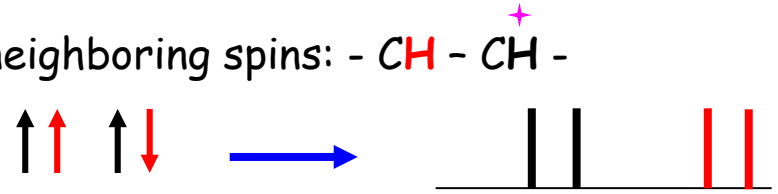
Example of I=1/2 nuclei

•N neighboring spins: split into N + 1 lines

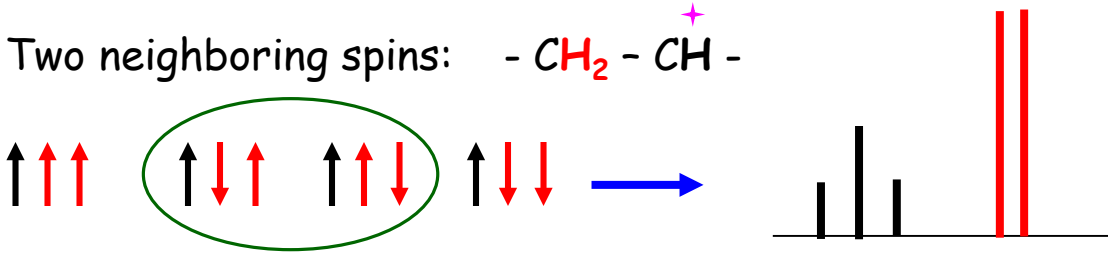
Single spin:



One neighboring spins: - CH – CH -



Two neighboring spins: - CH_2 – CH -



1

1 1

1 2 1

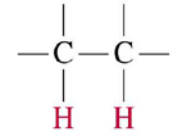
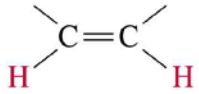
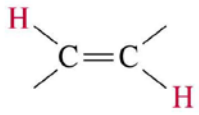
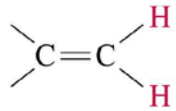
1 3 3 1

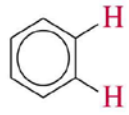
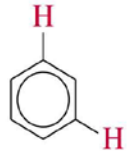
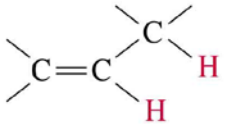
1 4 6 4 1

1 5 10 10 5 1

•Distance between the peaks of multiple is measured in Hz

•Coupling constant does not dependent on strength of the external field

		Approx. J
	(free rotation)	7 Hz[a]
	(cis)	10 Hz
	(trans)	15 Hz
	(geminal)	2 Hz

		Approx. J
	(ortho)	8 Hz
	(meta)	2 Hz
	(allylic)	6 Hz

[a]The value of 7 Hz in an alkyl group is averaged for rapid rotation about the carbon–carbon bond. If rotation is hindered by a ring or bulky groups, other splitting constants may be observed.

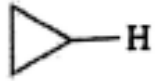
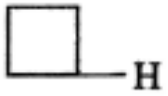
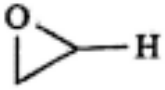
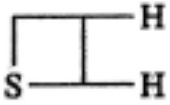
Example of H-X Coupling Constant

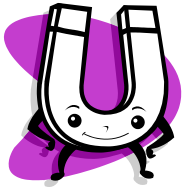
- Spin-spin coupling takes place between all NMR active nuclei, not just between protons. (EX: coupling to ^{13}C, ^{31}P, ^{19}F… but many other nuclei can couple.)
- Coupling constant does not dependent on strength of the external field

$^{1}J_{XY}$

J_{CD}	20
J_{CF}	-167
J_{CN}	6-8
J_{PH}	>600

^{1}H-^{13}C

Type	J (Hz)	Type	J (Hz)
CH_3-H	125	CH_3Li	98
Ph-CH_2-H	129	Cl_2CH-H	178
RC≡C-CH_2-H	132	O_2N-CH_2-H	147
R_2NCH_2-H	133	FCH_3-H	149
$RSCH_2$-H	138	$ClCH_2$-H	150
$ROCH_2$-H	140	ICH_2-H	151
$(NC)_2CH$-H	145	$BrCH_2$-H	152
	161	$(CH_3O)_2CH$-H	162
	134		180
	137		137
	150		150



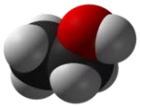
NMR could be helpful for diverse applications

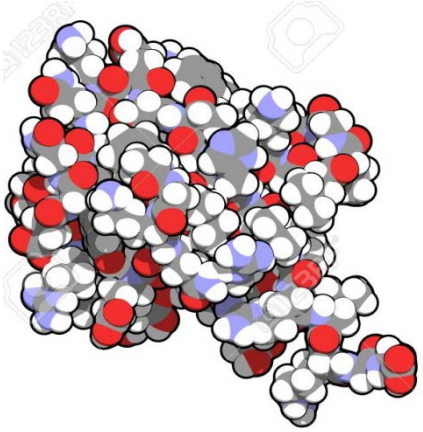
◆Due to different chemical or physical environment of the atom in the molecule, as well as the difference in atom number, NMR signals would be different !!

原子核週遭環境只要有一點不同, 核磁共振訊號就不同 !!

原子核個數不同, 訊號強度也不同!!

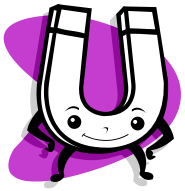
H2O 

C2H5OH 

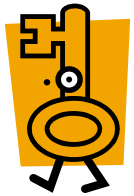
Ubiquitin 

◆Therefore, NMR could provide detail atomic structural information for molecules under specific condition, or provide useful information on molecular interaction studies.

不同的分子, 乃由原子以各種排列方式組合而成. 因此核磁共振方法是用來研究分子結構/狀態的重要工具之一, 對於”分子間作用”的相關課題也有極大的助益!!



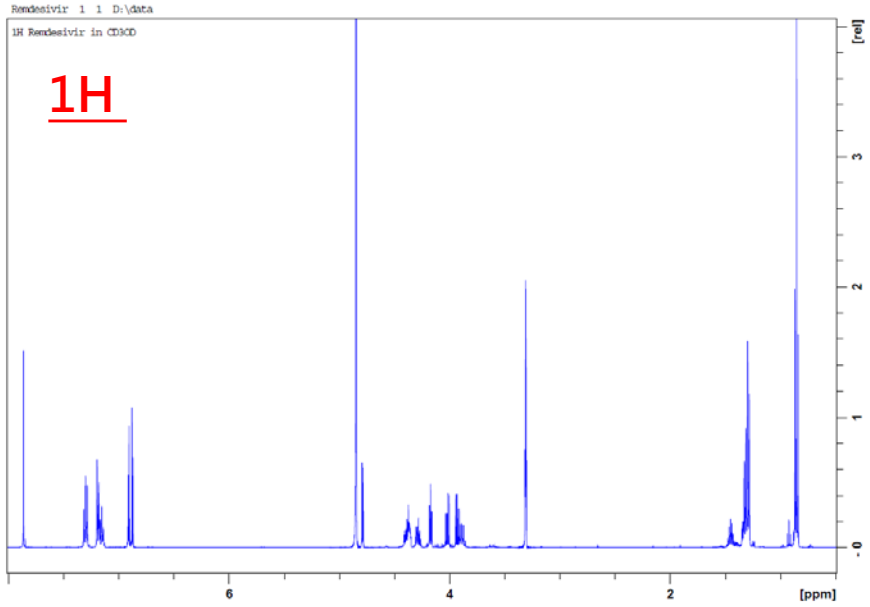
Applications for NMR in HFNMRC & GRC

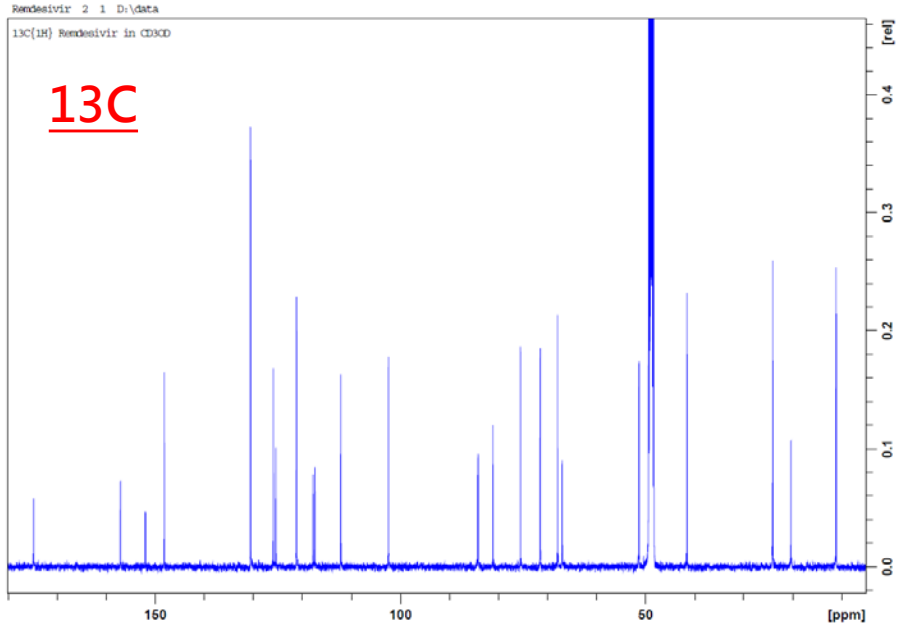


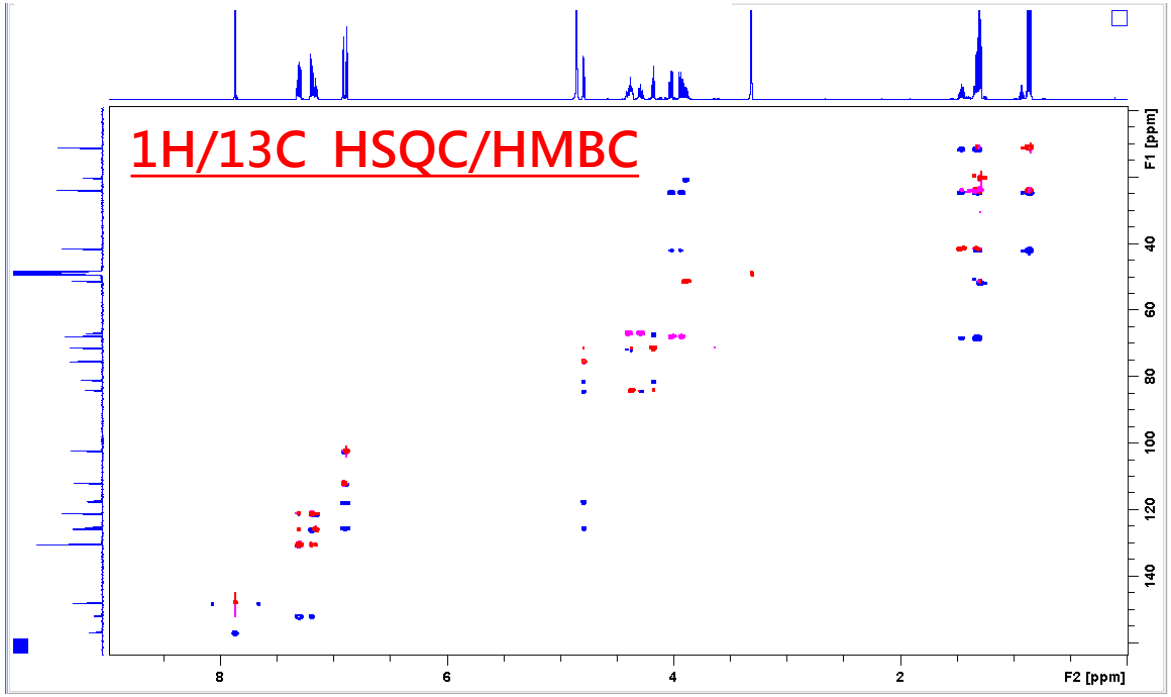
- ✓ Structure identification/ elucidation for small molecules, nature products……
- ✓ Bimolecular structure /Dynamics (ex: nucleic acids, peptides, proteins)
- ✓ Molecule-Molecule interaction (protein-ligand, protein-protein, protein-DNA ….)
- ✓ Fragment Based Drug Screening
- ✓ In cell NMR / Metabolomic NMR
- ✓ Others (qNMR….)

Applications of NMR Spectroscopy

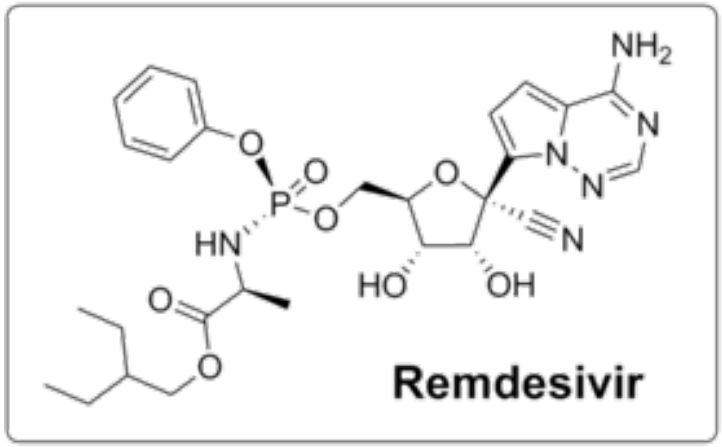
Example 1:
Structure identification







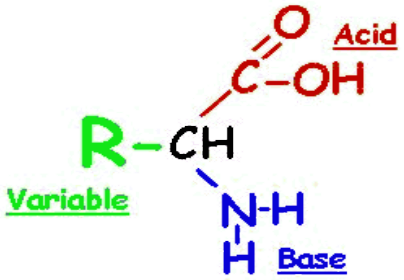
Structure confirmed by NMR



Applications of NMR Spectroscopy

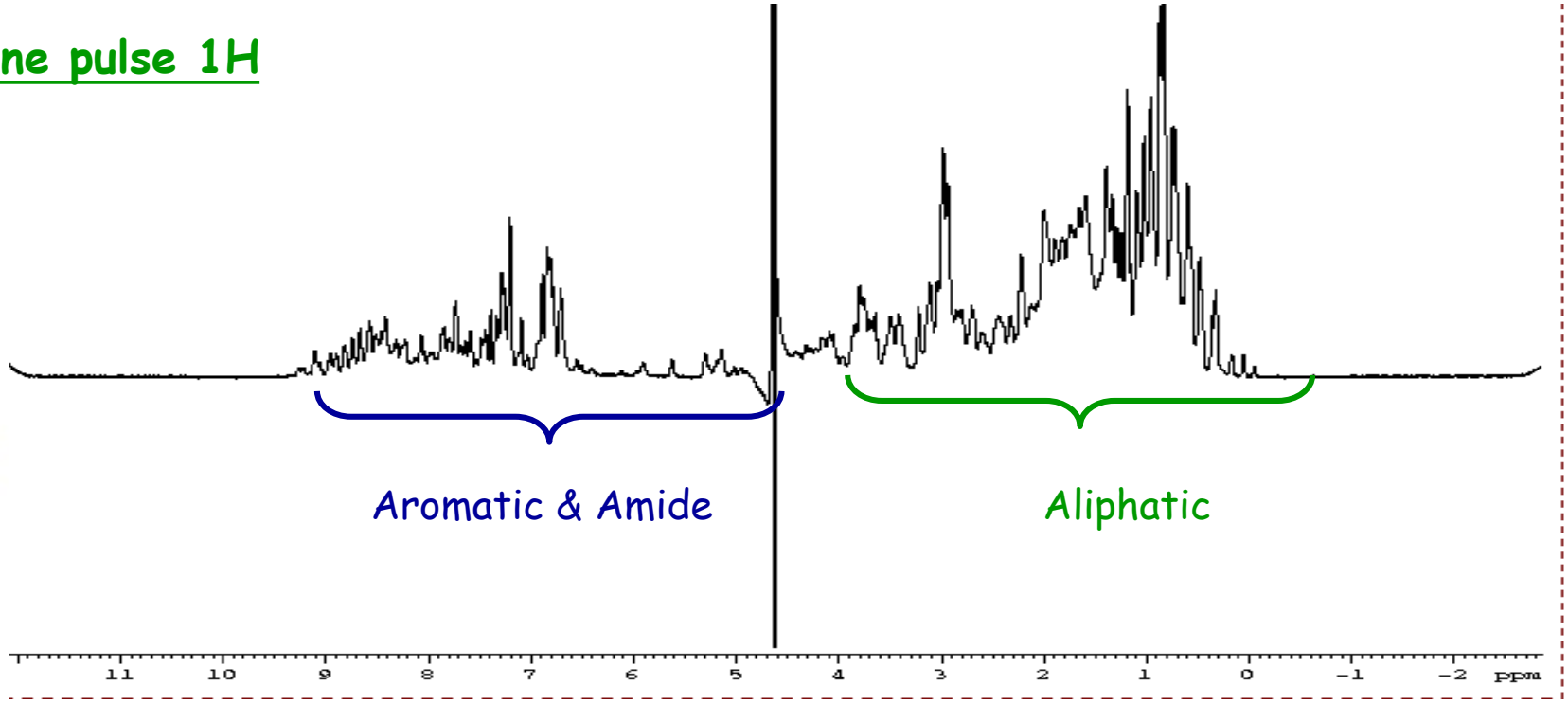
Example 2:
Biomolecule Structure/Dynamics

Amino Acids to Proteins

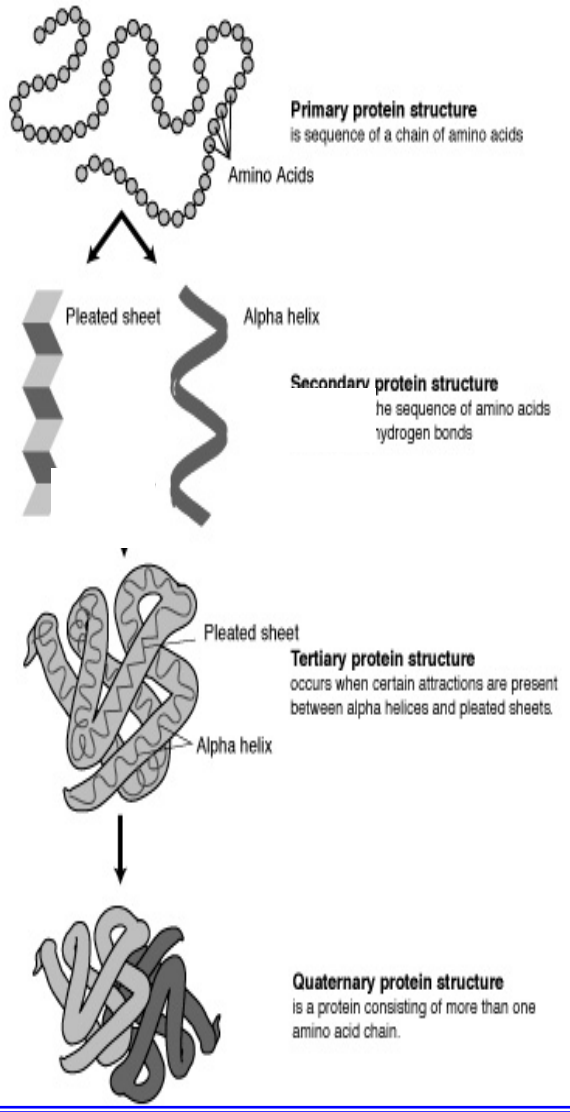


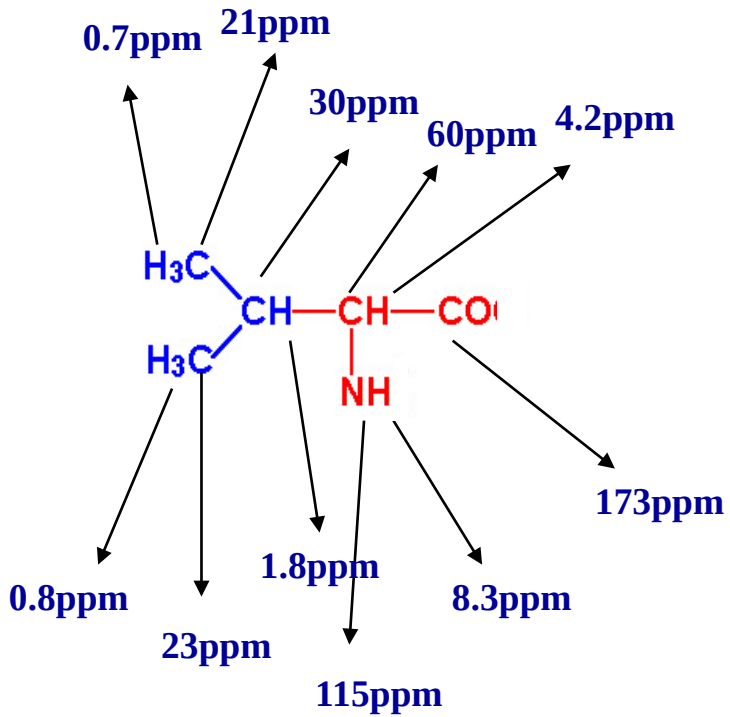
A: Ala, **C**: Cys, **D**: Asp, **E**: Glu, **F**: Phe, **G**: Gly, **H**: His,
I: Ile, **K**: Lys, **L**: Leu, **M**: Met, **N**: Asn, **P**: Pro, **Q**: Gln,
R: Arg, **S**: Ser, **T**: Thr, **V**: Val, **W**: Trp, **Y**: Tyr

1D one pulse 1H



Sequence-specific and total resonance assignment





Try to assign Protein residues chemical shift as many as possible

Protein in solution 0.3~0.5ml, ~0.1~1mM

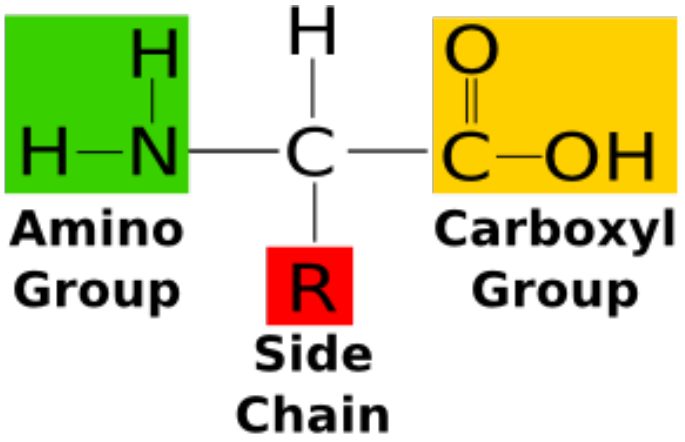
- Non-labeled
- 15N labeled
- 13C/15N labeled
- 2H/15N labeled
- 2H/15N/13C labeled
- Selected & Other specific labeled

Protein Size Limitation :

(1) Slow tumbling problem

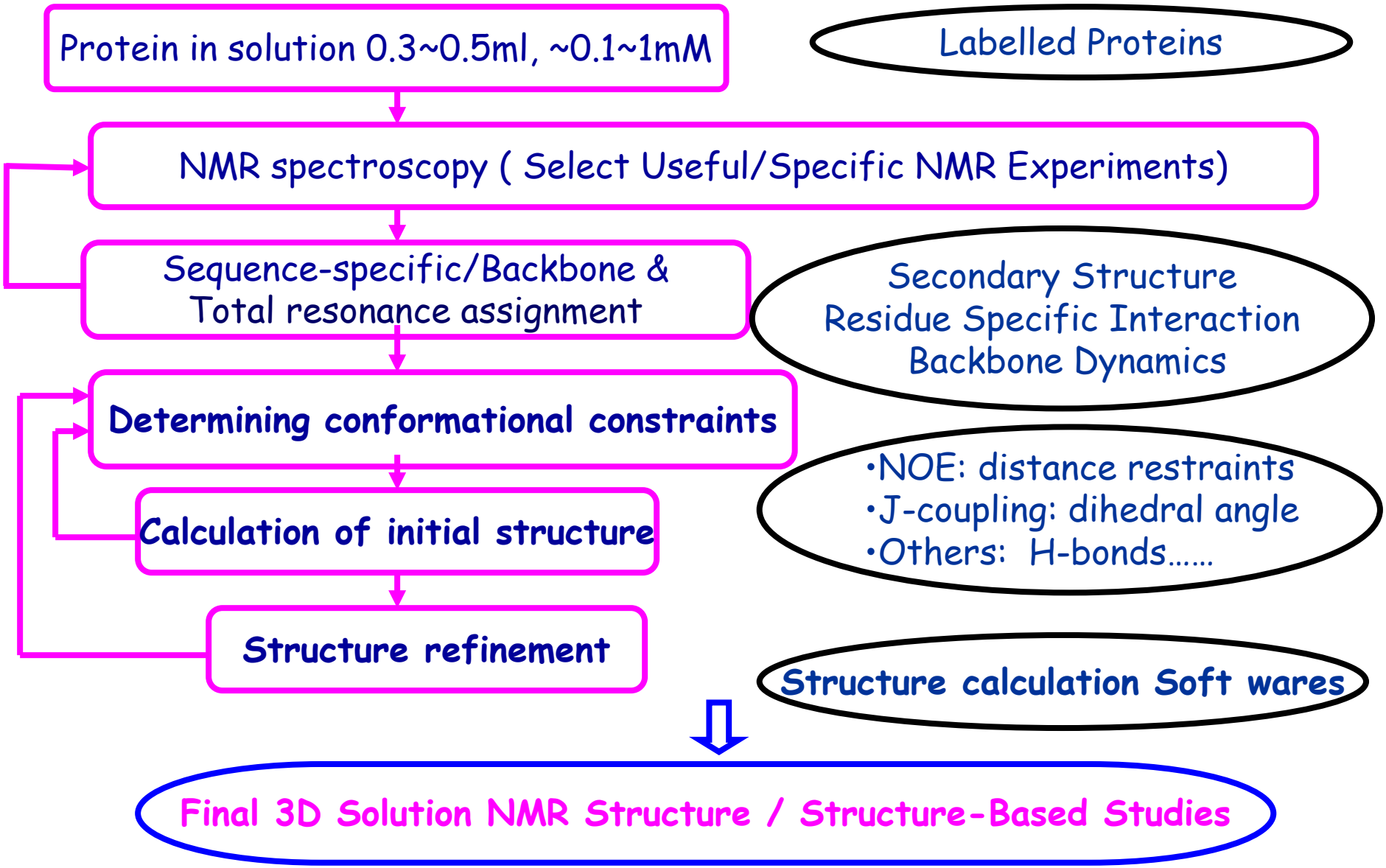
(2) Signal overlapping problem

13C: Nature abundance 1.1 %
15N: Nature abundance 0.4%

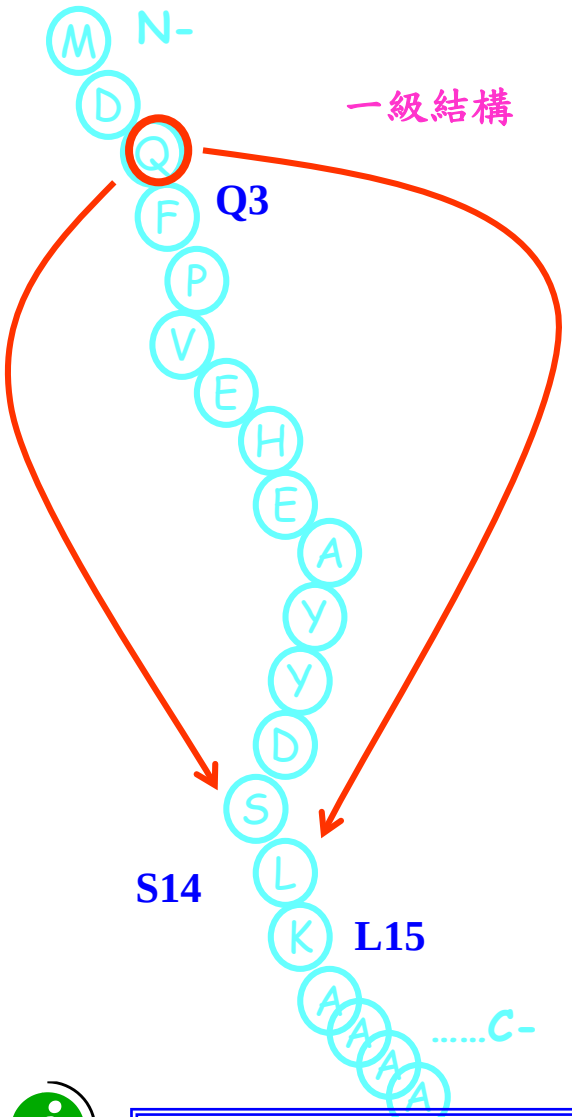


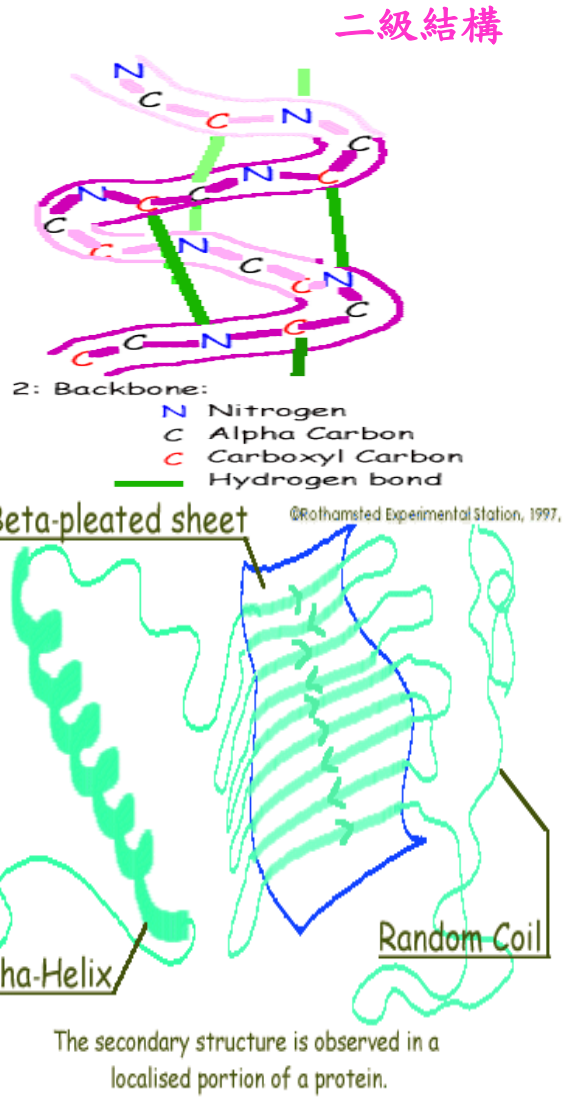
Understand the limitation, find the best condition!

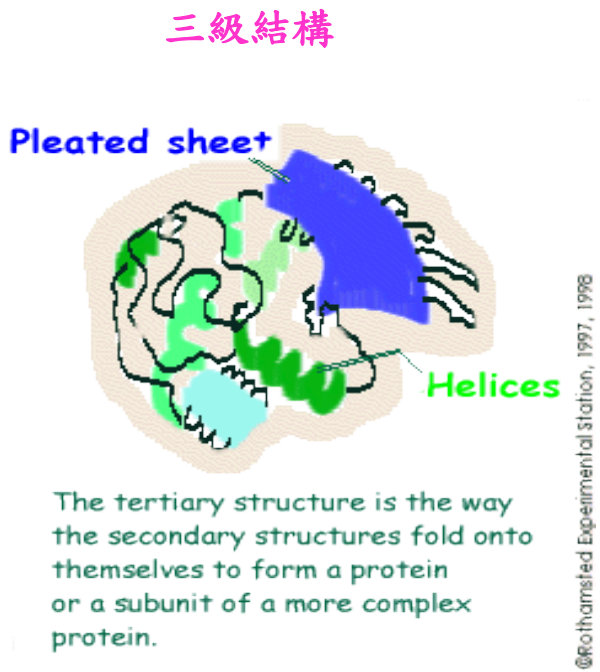
Workflow for Protein NMR Studies



Determine conformational constraints



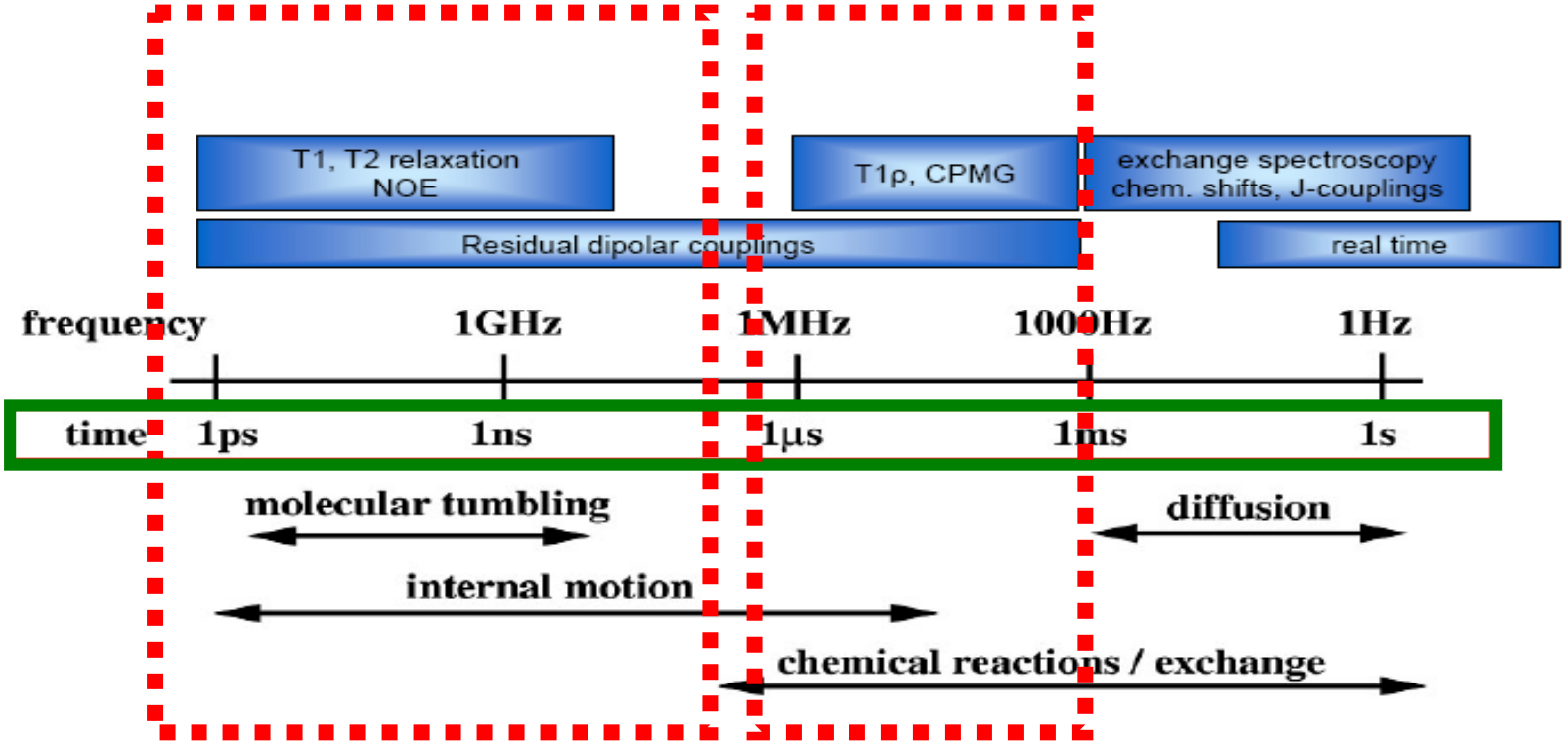


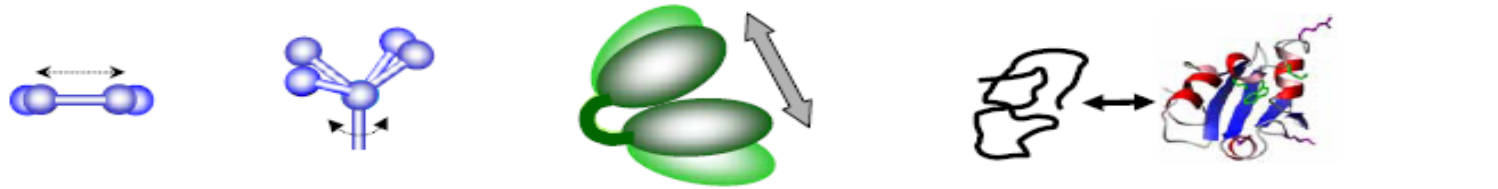


Obtain "distance or angle " information between atoms or bonds

NMR Time Scales for Protein Dynamics Studies

(From http://www.embl.de/nmr/sattler/teaching)





bond vibrations | *side chain rotations* | *domain movements* | *enzyme kinetics* | *folding, H/D exchange*

Applications of NMR Spectroscopy

Example 3:
Molecule-Molecule interaction
/Fragment-based screening by NMR

Our approach for NMR screening

"Free" Compound Groups

- 1D 1H spectrum
- T1ρ spectrum

- 0.2mM compounds
- PBD or d18-Tris Buffer @pH7.0

Compound Groups + Target Protein

- 1D 1H spectrum
- T1ρ spectrum
- WaterLOGSY spectrum
- STD spectrum

- 0.2mM compounds + ~0.01mM Protein
- PBD or d18-Tris Buffer @pH7.0

Find the " Hit Groups"

Analyze all spectrum
Free vs. Complex

Narrow down to "individual Hits"

Our approach for NMR screening

"Free" Protein NMR → ·Backbone Assignments ·15N labelled protein

- 0.1mM 15N-labelled Protein
- PBD or d18-Tris Buffer @pH7.0

↓

Target Protein + Hit Compounds → ·1D 1H spectrum ·2D 15N-1H HSQC

- ~0.1mM Protein + compounds (titration)
- PBD or d18-Tris Buffer @pH7.0

↓

Titration to find " Activity Site" → Analyze Chemical Shift perturbation Free vs. Complex

↓

SAR (structure-activity relationship) by NMR → Provide information for Compound Optimization

Applications of NMR Spectroscopy

Example 4:
Metabolomics

Human related Metabolomics NMR Studies

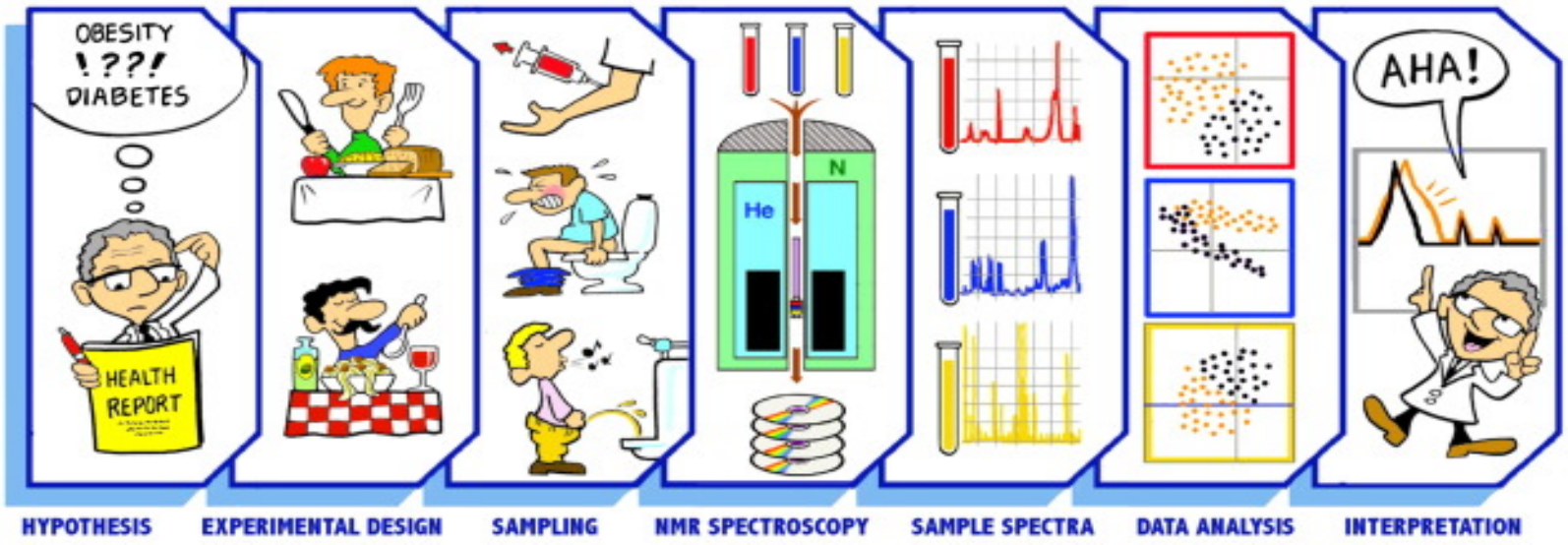


Fig from Food Research International 54 (2013) 1131–1145

Plant Metabolomics Studies

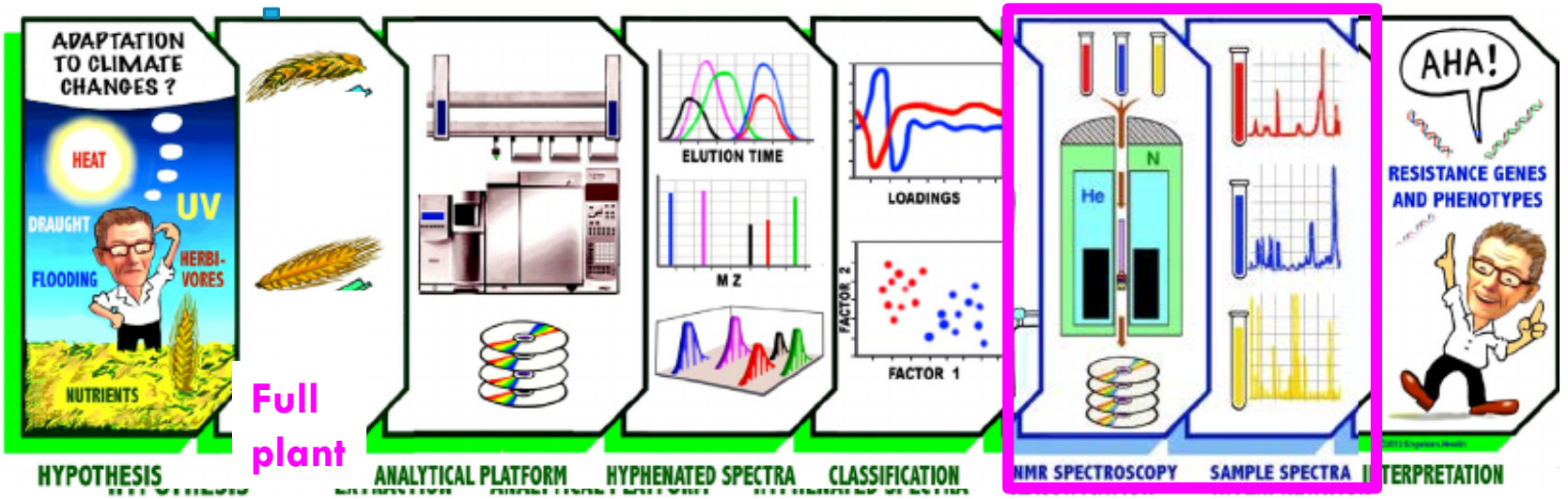


Fig from Journal of Cereal Science Volume 59, Issue 3, 393-418

Different Techniques are Needed for Research
NMR can provide information @ atomic resolution