Fundamentals of Protein NMR Spectroscopy

by
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$$\rho_0 = I_z \xrightarrow{\mathcal{H}} 2I_z S_y \xrightarrow{\mathcal{H}} I_x \xrightarrow{\mathcal{H}} S(t_1, t_2)$$
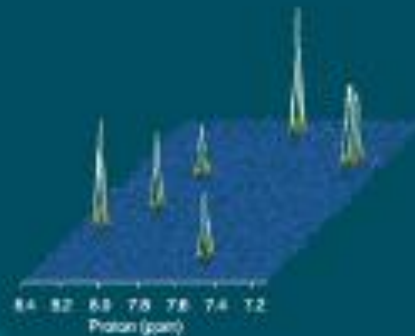
Focus on Structural Biology

	1nm	10	10^2	10^3	10^4	10^5	10^6	10^7
(The wave)		X-ray		UV/VIS		Infrared		Microwave	RF
(The transition)			**Electronic**		**Vibration**		**Rotation**	**Nuclear**
(Spectroscopy)		X-ray		UV/VIS	Infrared/Raman		ESR	NMR
					Fluorescence			
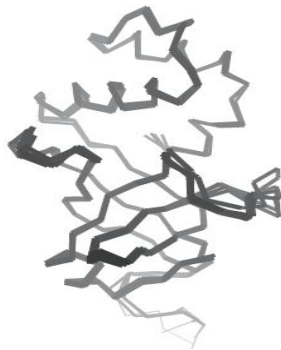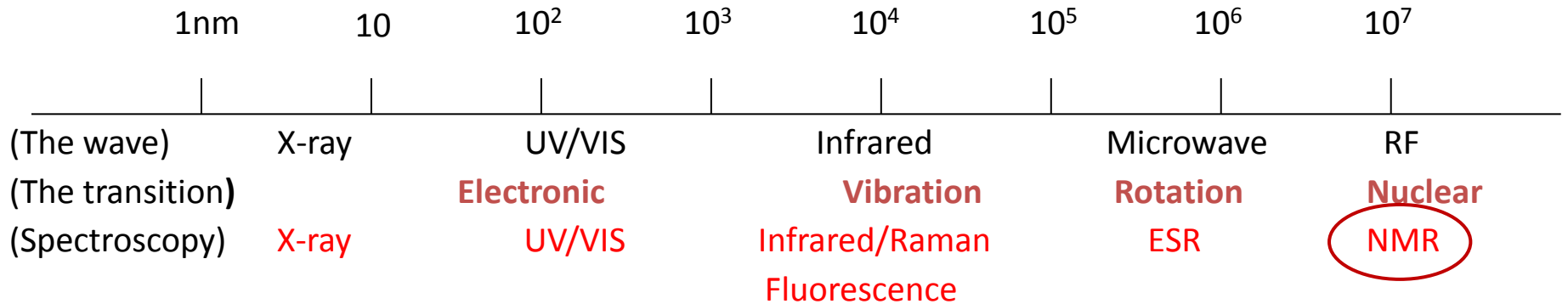
➤ **Structure**
➤ **Dynamics**
➤ **Interactions**

NMR method -------

Time scale --------

Molecular motion --

Spin Relaxation

Residual dipolar coupling ——————→

Chemical exchange

Amide hydrogen exchange

–11	–10	–9	–8	–7	–6	–5	–4	–3	–2	–1	0

Methyl rotation   Loop dynamics   Aromatic ring flips   ←—— Protein domain motion ——→
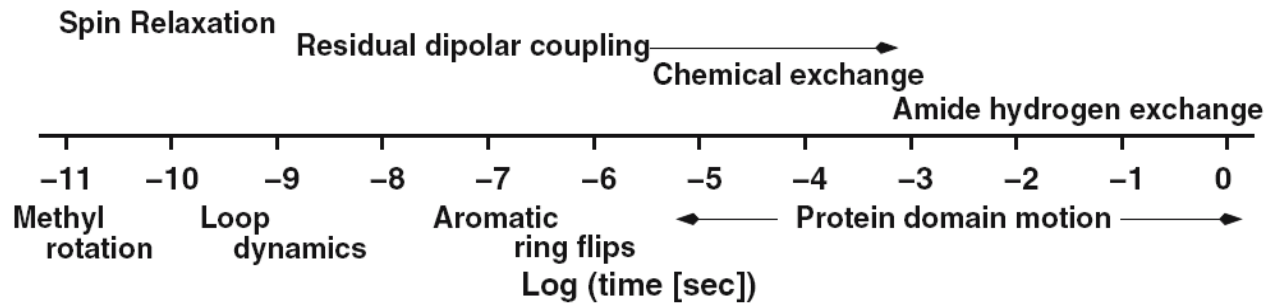
Log (time [sec])

Figure 1.2. Characterization of molecular dynamics with NMR. The time scale of motions in proteins are indicated below the axis and NMR techniques that can be used to characterize motion on the various time scales are shown above the axis.

1.1   Introduction to NMR Spectroscopy

In this chapter we will employ a semi-classical model of the nuclear spins to obtain an intuitive understanding of many of the fundamental aspects of modern NMR spectroscopy. This treatment will highlight a number of important features of NMR spectroscopy, including:

1. How energy states are created by the magnetic field,
2. How resonance signals are detected,
3. How the detected signals are transformed into spectra,
4. How the relaxation properties of the excited state are affected by the environment,
5. How the relaxation properties affect the NMR lineshape,
6. How the absorption frequency of a nuclear spin is affected by its environment,
7. How the characteristic NMR absorption frequencies of amino acids are interpreted.

1.2   One Dimensional NMR Spectroscopy
1.2.1   Classical Description of NMR Spectroscopy
1.2.2.1   Magnetic Dipole

Magnetic moment of a nuclear spin: $\vec{\mu}_n = \gamma_n \hbar \vec{I}$ (Erg/Gauss)

γ : Nuclear gyric ratio (Gyromagnetic ratio) (Hz/gauss)
$\hbar$: Plank's constant (Erg/Hz);   I: Spin quantum number (unitless)

γ and I are intrinsic properties of a nucleus

Table of Isotopes and NMR Parameters

NMR Observation Frequencies for GX-series

Isotope	Observation Frequency			Natural Abundance	Spin(I)	Relative Sensitivity
	GX270	GX400	GX500			
※ 1H	270.166	399.782	500.125	99.985	1/2	1.00
2D	41.472	61.369	76.773	1.5×10^{-2}	1	9.65×10^{-3}
3T	288.168	426.420	533.449		1/2	1.21
6Li	39.758	58.832	73.598	7.42	1	8.50×10^{-3}
※ 7Li	105.014	155.396	194.400	92.58	3/2	0.293
9Be	37.962	56.175	70.275	100	-3/2	1.39×10^{-2}
10B	29.032	42.960	53.743	19.58	3	1.99×10^{-2}
※ 11B	86.677	128.262	160.455	80.42	3/2	0.165
※ 13C	67.938	100.533	125.766	1.108	1/2	1.59×10^{-2}
※ 14N	19.519	28.884	36.134	99.63	1	1.01×10^{-3}
※ 15N	27.379	40.514	50.683	0.37	-1/2	1.04×10^{-3}
※ 17O	36.634	54.210	67.817	3.7×10^{-2}	-5/2	2.91×10^{-2}
※ 19F	254.191	376.142	470.552	100	1/2	0.833
※ 23Na	71.458	105.742	132.282	100	3/2	9.25×10^{-2}
※ 25Mg	16.538	24.472	30.615	-10.13	-5/2	2.67×10^{-3}
※ 27Al	70.396	104.169	130.315	100	5/2	0.206
※ 29Si	53.674	79.426	99.361	4.70	-1/2	7.84×10^{-2}
※ 31P	109.381	161.858	202.483	100	1/2	6.63×10^{-2}
※ 33S	21.039	31.133	38.948	0.76	3/2	2.26×10^{-3}
※ 35Cl	26.471	39.170	49.002	75.53	3/2	4.70×10^{-3}
37Cl	22.032	32.602	40.785	24.47	3/2	2.71×10^{-3}
39K	12.606	18.654	23.336	93.10	3/2	5.08×10^{-4}
41K	6.919	10.238	12.808	6.88	3/2	8.40×10^{-5}
※ 43Ca	17.911	26.504	33.156	0.145	-7/2	6.40×10^{-3}
45Sc	65.631	97.119	121.495	100	7/2	0.301
47Ti	15.229	22.536	28.192	7.28	-5/2	2.09×10^{-3}
49Ti	15.232	22.540	28.197	5.51	-7/2	3.76×10^{-3}
50V	26.936	39.858	49.862	0.24	6	5.55×10^{-2}
51V	71.008	105.075	131.448	99.76	7/2	0.383
53Cr	15.270	22.596	28.267	9.55	-3/2	9.03×10^{-4}
55Mn	66.634	98.602	123.351	100	5/2	0.175
57Fe	8.729	12.917	16.159	2.19	1/2	3.37×10^{-5}
※ 59Co	64.106	94.862	118.672	100	7/2	0.277
61Ni	24.142	35.724	44.691	1.19	-3/2	3.57×10^{-3}
※ 63Cu	71.607	105.961	132.557	69.09	3/2	9.31×10^{-2}
65Cu	76.711	113.514	142.005	30.91	3/2	0.114
67Zn	16.899	25.006	31.283	4.11	5/2	2.85×10^{-3}
69Ga	64.840	95.948	120.030	60.4	3/2	6.91×10^{-2}
※ 71Ga	82.387	121.914	152.514	39.6	3/2	0.142
73Ge	9.423	13.944	17.444	7.76	-9/2	1.40×10^{-3}
※ 75As	46.281	68.484	85.673	100	3/2	2.51×10^{-2}
※ 77Se	51.525	76.245	95.382	7.58	1/2	6.93×10^{-3}
79Br	67.687	100.161	125.301	50.54	3/2	7.86×10^{-2}
※ 81Br	72.980	107.993	135.099	49.46	3/2	9.85×10^{-2}
83Kr	10.393	15.380	19.240	11.55	-9/2	1.88×10^{-3}
85Rb	26.085	38.599	48.287	72.15	5/2	1.05×10^{-2}
※ 87Rb	88.403	130.815	163.649	27.85	3/2	0.175
87Sr	11.706	17.323	21.670	7.02	-9/2	2.69×10^{-3}
89Y	13.235	19.585	24.501	100	-1/2	1.18×10^{-4}
91Zr	25.206	37.300	46.662	11.23	-5/2	9.48×10^{-3}
※ 93Nb	66.036	97.717	122.244	100	9/2	0.482
95Mo	17.061	26.046	32.583	15.72	5/2	3.23×10^{-3}
97Mo	17.971	26.593	33.268	9.46	-5/2	3.43×10^{-3}
99Ru	9.133	13.515	16.907	12.72	-3/2	1.95×10^{-4}
101Ru	13.241	19.594	24.511	17.07	-5/2	1.41×10^{-3}
103Rh	8.505	12.585	15.744	100	-1/2	3.11×10^{-5}
105Pd	12.371	18.306	22.901	22.23	-5/2	1.12×10^{-3}
107Ag	10.934	16.179	20.240	51.82	-1/2	6.62×10^{-5}
109Ag	12.568	18.598	23.266	48.18	-1/2	1.01×10^{-4}
111Cd	57.286	84.770	106.047	12.75	-1/2	9.54×10^{-3}
※ 113Cd	59.926	88.676	110.933	12.26	-1/2	1.09×10^{-2}
113In	59.069	87.408	109.347	4.28	9/2	0.345
115In	59.204	87.608	109.597	95.72	9/2	0.342
115Sn	88.342	130.725	163.536	0.35	-1/2	3.50×10^{-2}
117Sn	96.249	142.426	178.175	7.61	-1/2	4.52×10^{-2}
※ 119Sn	100.682	148.985	186.380	8.58	-1/2	5.18×10^{-2}
121Sb	64.653	95.672	119.685	57.25	5/2	0.160
123Sb	98.467	145.708	182.281	42.75	7/2	4.57×10^{-2}
123Te	70.812	104.785	131.086	0.87	-1/2	1.80×10^{-2}
125Te	85.348	126.295	157.994	6.99	-1/2	3.15×10^{-2}
※ 127I	54.062	79.999	100.078	100	5/2	9.34×10^{-2}
129Xe	74.731	110.584	138.340	26.44	1/2	2.12×10^{-2}
※ 133Cs	35.443	52.448	65.612	100	7/2	4.74×10^{-2}
135Ba	26.838	39.714	49.682	6.59	3/2	4.90×10^{-3}
137Ba	30.024	44.428	55.579	11.32	3/2	6.86×10^{-3}
139La	38.164	56.473	70.648	99.911	7/2	5.92×10^{-2}
141Pr	79.459	117.581	147.094	100	5/2	0.293
143Nd	14.689	21.736	27.192	12.17	-7/2	3.38×10^{-3}
145Nd	9.024	13.353	16.704	8.3	-7/2	7.86×10^{-4}
147Sm	11.158	16.511	20.655	14.97	-7/2	1.48×10^{-3}
149Sm	8.890	13.156	16.457	13.83	-7/2	7.47×10^{-4}
151Eu	66.990	99.130	124.011	47.82	5/2	0.178
153Eu	29.589	43.784	54.774	52.18	5/2	1.53×10^{-2}
155Gd	10.253	15.172	18.980	14.73	-3/2	2.79×10^{-4}
159Tb	61.315	90.731	113.504	100	3/2	5.83×10^{-2}
161Dy	8.904	13.175	16.482	18.88	-5/2	4.17×10^{-4}
163Dy	12.681	18.765	23.475	24.97	5/2	1.12×10^{-3}
165Ho	55.379	81.948	102.516	100	7/2	0.181
167Er	7.825	11.578	14.485	22.94	-7/2	5.07×10^{-4}
169Tm	22.327	33.039	41.331	100	-1/2	5.66×10^{-4}
171Yb	47.584	70.414	88.087	14.31	1/2	5.46×10^{-3}
173Yb	13.108	19.397	24.266	16.13	-5/2	1.33×10^{-3}
175Lu	30.826	45.615	57.064	97.41	7/2	3.12×10^{-2}
177Hf	8.375	12.393	15.504	18.5	7/2	6.38×10^{-4}
179Hf	5.126	7.586	9.490	13.75	-9/2	2.16×10^{-4}
181Ta	32.337	47.851	59.861	99.988	7/2	3.60×10^{-2}
183W	11.242	16.635	20.810	14.40	1/2	7.20×10^{-5}
185Re	60.825	90.007	112.598	37.07	5/2	0.133
187Re	61.449	90.930	113.753	62.93	5/2	0.137
187Os	6.222	9.207	11.518	1.64	1/2	1.22×10^{-5}
189Os	21.051	31.151	38.970	16.1	3/2	2.34×10^{-3}
191Ir	4.644	6.872	8.597	37.3	3/2	2.53×10^{-5}
193Ir	5.055	7.480	9.357	62.7	3/2	3.27×10^{-5}
※ 195Pt	58.077	85.941	107.511	33.8	1/2	9.94×10^{-3}
197Au	4.625	6.844	8.562	100	3/2	2.51×10^{-5}
※ 199Hg	48.308	71.484	89.426	16.84	1/2	5.67×10^{-3}
201Hg	17.831	26.386	33.008	13.22	-3/2	1.44×10^{-3}
203Tl	154.400	228.475	285.821	29.50	1/2	0.187
205Tl	155.910	230.710	288.617	70.50	1/2	0.192
※ 207Pb	56.534	83.657	104.655	22.6	1/2	9.16×10^{-3}
209Bi	43.416	64.245	80.370	100	9/2	0.137
235U	4.863	7.196	9.002	0.7205	7/2	1.21×10^{-4}

※Isotopes whose observation frequencies are stored on GX standard software.

For biological systems we deal almost exclusively with ^1H, ^2H, ^{13}C, ^{15}N and ^{31}P. With the exception of 2H they are all spin $\frac{1}{2}$ nuclei.

Table 1.1. Properties of NMR active nuclei.

Nuclei[1]	γ (rad $\times$ sec$^{-1}\times$ gauss^{-1})[†]	I	Natural Abundance (%)
^1H	26,753	1/2	99.980
^2H	4,106	1	0.016
^{19}F	25,179	1/2	100.000[2]
^{13}C	6,728	1/2	1.108[3]
^{15}N	-2,712	1/2	0.37[3]
^{31}P	10,841	1/2	100.00

[1] The term "Protons" is used interchangeably with ^1H in the text.
[2] Fluorine is not normally found in biopolymers, therefore it has to be introduced by chemical or biosynthetic labeling.
[3] These isotopes of carbon and nitrogen are normally found in low levels in biopolymers, therefore the levels of these two spins are generally enriched, often to 100%, by biosynthetic labeling.
[†] CGS units are used throughout the text.

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(1T = 10,000G)

W_0(MHz)	0	30	75	121		280	300	320
Nucleus		↑ ^{15}N	↑ ^{13}C	↑ ^{31}P		↑ ^{19}F	↑ ^1H	↑ ^3H

1.2.2.2   Nuclear Dipole-Magnetic Field Interaction
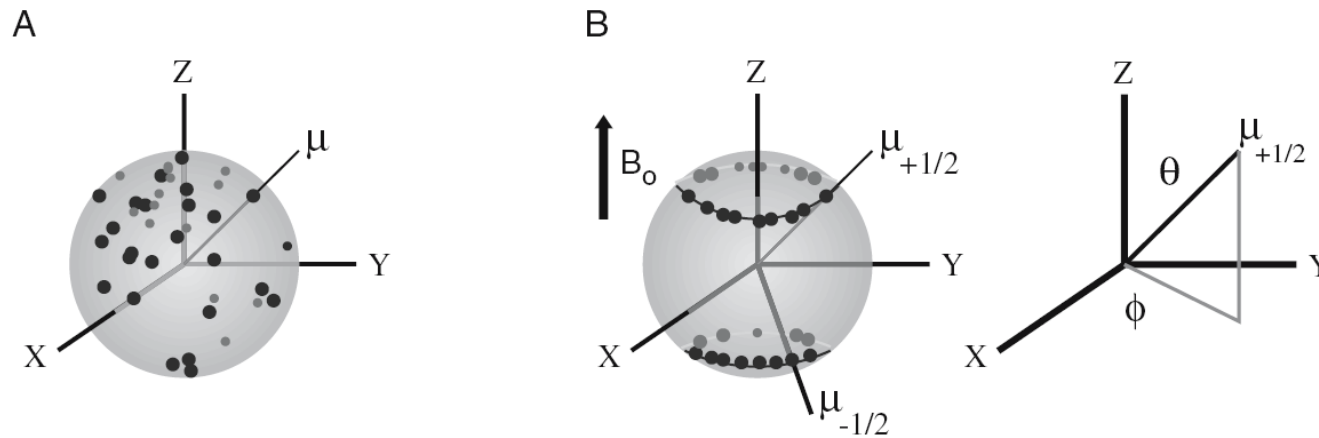
Figure 1.3.   Orientation of magnetic dipoles.
A. Orientation of nuclear magnetic dipoles in the absence of a magnetic field. A unit sphere is shown and the dots on the surface illustrate the various orientations of the dipoles in space. The orientation of one dipole μ is indicated by a line drawn from the center of the sphere.
B. The orientation of the nuclear spin dipoles in a static magnetic field along the z-axis. Note that approximately one-half of the spins are pointed up and the other half are pointed down. Also note that they can assume any value of ϕ, but only two values of θ. ϕ and θ represent the orientation of the magnetic dipole in spherical coordinates, as shown on the right part of this figure.

Energy of a spin in a magnetic field of strength B:

$$E = -\vec{\mu} \cdot \vec{B} \qquad\qquad B = (1 - \sigma)B_o$$

Where σ represents the degree of shielding due to surrounding electrons. Σ is in the order of 10^{-6} and will be ignored for the time being.

The Zeeman Hamiltonian: $\mathcal{H} = -u_z B_z = -\gamma\hbar B m_z$ for $\mu_z = \gamma\hbar m_z$

m_z = -I, -I+1, ...I-1, I $\qquad E_\alpha = -\dfrac{\gamma\hbar B}{2} \qquad E_\beta = +\dfrac{\gamma\hbar B}{2}$

$\Delta E = E_\beta - E_\alpha = \gamma\hbar B \qquad\qquad E = \hbar\omega$

$$\boxed{\omega_s = \gamma B \qquad \textbf{\textcolor{red}{Larmor equation}}}$$
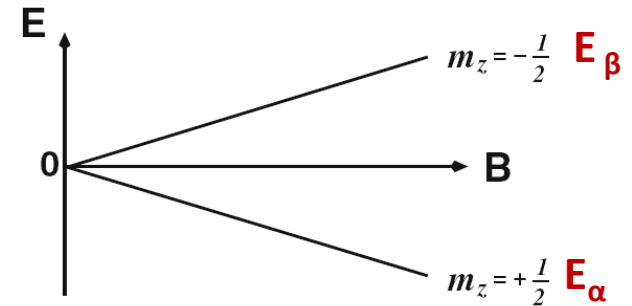
➢ Resonance frequency depends on:
(i) The type of nucleus, γ, and (ii) External field strength, B.

1. The population difference between the two energy levels is very small, on the order of 1 part in 10^6. The actual population difference can be easily calculated from Boltzmann's relationship:

$$\frac{N_\beta}{N_\alpha} = e^{\frac{-\gamma\hbar B}{kT}} \approx 1 - \frac{\gamma\hbar B}{kT} \tag{1.10}$$

➢ The higher the resonance frequency the more sensitive it is.

2. The lifetime of the excited state can be quite long, on the order of msec to sec. As discussed above, a long lifetime provides three benefits: narrow resonance lines, experimental manipulation of the excited state in multi-dimensional experiments, and sensitivity to molecular motion over a wide time scale.

1.3   Detection of Nuclear Spin Transitions

Side View

➤ The presence of static field caused the spins to occupy different energy states

➤ To detect the magnetization one need to apply a RF field of frequency equal to the Larmor frequency in the orthogonal direction to the static field.

B_0

Top View

Sample

B_1

Excitation/receive coil

Helmhotz coil
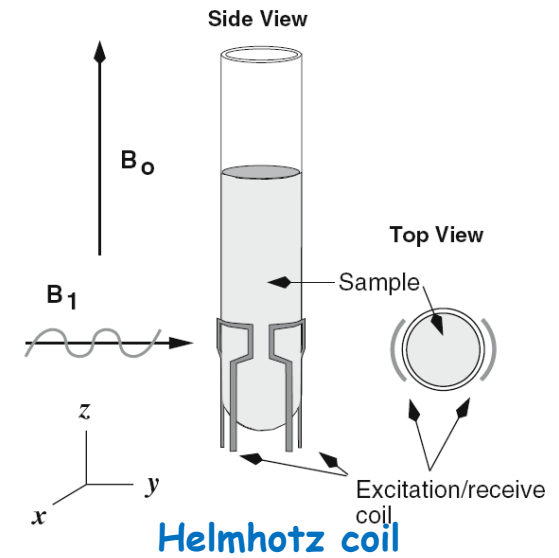
Two ways of detecting the magnetization:

1. Continuous wave method: Swapping the static or RF field so that the Larmor condition is met to observe absorption of RF field.
   ➜ Slow and not versatile.

Radio–frequency pulse

Signal (FID)

2. Pulsed Fourier Transform NMR:
   ➜ Fast (x100) and more versatile.
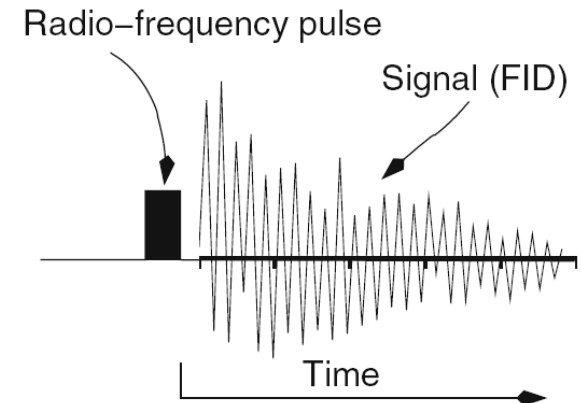
Time

Steps involved in a simple one pulse expt:
1. Preparation: Allow spin to reach thermal equilibrium.
2. Excitation: Apply B1 field at defined frequency and time duration.
3. Detection: B1 off and receiver on. The signal (Free induction decay, FID) is digitized and Fourier transformed to obatain spectrum.

1.3.2.1　Before the Pulse: Magnetization at Equilibrium

The torque on the magnetization subjecting to a magnetic field B is given by:

$$\Gamma = \frac{dS}{dt} = \vec{\mu} \times \vec{B} \qquad \text{Using } \vec{\mu} = \gamma \vec{S} \text{ we can write} \qquad \frac{d\mu}{dt} = \gamma \vec{\mu} \times \vec{B} \qquad (1.12)$$

Rotating frame: The change in magnetization as observed in a frame rotating a frequence Ω is given by:

$$\frac{\delta \vec{\mu}}{\delta t} = \gamma \vec{\mu} \times (\vec{B} + \frac{\Omega}{\gamma}) \qquad = \gamma \mu \times B_{eff} \qquad (1.14)$$

Where B_{eff} = $(\vec{B} + \frac{\Omega}{\gamma})$ is the effective field in the Z-direction.

Figure 1.7. Rotating frame of reference. The coordinate system is rotating at a frequency $|\Omega|$ about the z-axis.

➜ In the rotating frame equatin 1.14 has the same form as (1.12) provide B is replaced by B_{eff}.
➜ The additional field Ω/γ is a fictitious field.
➜ $\delta\mu/\delta t = 0$ if B_{eff} = 0 or $\Omega = -\gamma B$
➜ In this frame the magnetization appears as stationary (i.e. no change)
➜ In a frame rotating at the Larmor frequency the magnetization appears as stationary (No oscillation).

The bulk (macroscopic magnetization:

$$M_i = \sum_{i=1}^{All\ spins} \mu_i$$

At thermal equilibrium the components in the three axes are:

$$M_z = M_o \qquad\qquad M_x = M_y = 0$$

1.3.2.2   Effect of the B_1 Pulse: Excitation of Nuclear Spins

If we apply a RF field $\vec{B_1} = |b_1|cos(\omega t)\hat{j}$ in the y-direction.

Then the effective in the rotation frame : $\vec{B}_{rot} = \left[(B + \frac{\Omega}{\gamma})\hat{k} + B_1\hat{j}\right]$

$$\frac{\delta\mu}{\delta t} = \gamma\mu \times \left[(B + \frac{\Omega}{\gamma})\hat{k} + B_1\hat{j}\right]$$

Let: $\Omega = -\omega; \gamma B = \omega_s; \omega_1 = \gamma B_1$, then

Then: $\frac{\delta\mu}{\delta t} = \gamma\mu \times \left[(\frac{\omega_s - \omega}{\gamma})\hat{k} + \frac{\omega_1}{\gamma}\hat{j}\right]$

Where ω_s is the resonance frequency of the nucleus, ω is the frequency of the applied RF field and ω_1 is proportional to B1 field strength.

➔ B and ω differ only by a factor γ and they can be considered as the same quantities.
➔ There are two fields, one in the Z-direction and is given by the static field and the opposing fictitious field and another one in the B1 direction (X or Y depending on the operator).

For the case ω_s = ω (on resonance) we have: $\frac{\delta\vec{\mu}}{\delta t} = \gamma\mu \times \frac{\omega_1}{\gamma}\hat{j}$

For the macroscopic magnetization: $\frac{\delta\vec{M}}{\delta t} = -\omega_1\hat{i}$
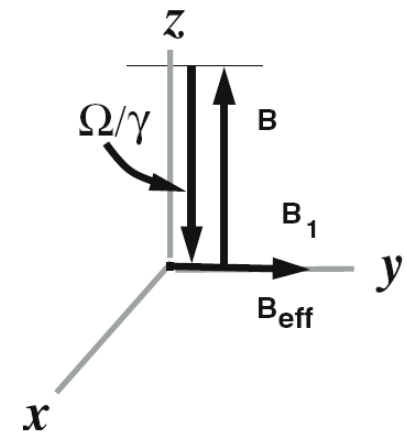
➔ M is tipped away from the z-axis at a rate of ω_1 rad/sec.
➔ The angle it tips: $\beta = \omega_1\tau$ where τ is the duration of the RF pulse.

Define $P_\beta^{\vec{u}}$ as a pulse in the $\vec{u}$ that flips the magnetization by β degree.

So P_{45}^y is a 45 y-pulse. If β = 90 it is called a 90º or π /2 pulse or a π-pulse if If β = 180º.

Magnetic Dipoles

After a $P_\beta{}^y$ pulse the magnetization has the following components:

$$M_z = M_o \cos(\beta) \qquad M_x = M_o \sin(\beta) \qquad M_y = 0$$

This transformation is often abbreviated as:

$$M_z \xrightarrow{\ P_\beta^y\ } M_z cos(\beta) + M_x sin(\beta)$$

Bulk Magnetic Moment

A π/2 pulse generates the maximum amountof magnetization in the x-y plane (maximum signal, transverse magnetization) while a π-pulse generates a –M magnetization and is called an inversion pulse.

➔ The distribution of magnetization after a π /2 pulse is called a ***coherent*** state.

➔ After a $P_\beta{}^{-y}$ pulse we have: $\quad M_z = M_o \cos(\beta) \qquad M_x = -M_o \sin(\beta)$

➔ A $P_\beta{}^x$ pulse will produce: $\ M_z = M_o \cos(\beta); \quad M_x = 0 \ $ and $\ M_y = - M_o\sin(\beta)$

➔ After a $P_{\pi/2}{}^y$ pulse: $M_z = 0; \ M_x = M_o \ $ and $\ M_y = 0 \ $ ➔ Magnetization on x-y plane.
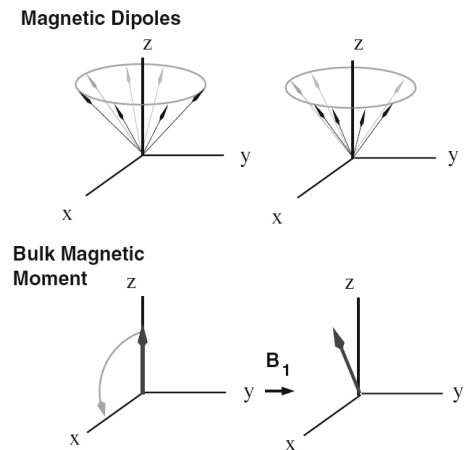
1.3.2.3 Detection of Resonance

After the B_1 pulse is turned off, the transverse magnetization precesses in the x-y plane around the B_o field, just as it did before the pulse. The key difference is that the transverse magnetization is now coherent and gives rise to a non-zero magnetic moment in the x-y plane.

The precession of the coherent magnetization in the x-y plane induces a time dependent current in the receiver coil. This signal is called the *free induction decay* (FID) and represents bulk magnetization that exists in the x-y plane. The frequency of the induced signal is *exactly* equal to the resonance frequency of the nuclear spin transition since the magnetization precesses around B_o at $\omega_s = \gamma B$.

Detection of the precessing magnetization is accomplished by analog circuits that actually measure the magnetization in the rotating frame, i.e. the observed frequency, ω', is $\omega_s - \omega$, where ω_s is the precessional frequency of the spin and ω is the rate of rotation of the coordinate frame, or equivalently, the frequency of the applied B_1 pulse.

a (ω' = 0, On resonance)
Envelope is given by $e^{-t/T2}$
c (ω' = 650 Hz)
b (ω' = 150 Hz)

FT

Question: What will it looks like if ω = - 150 Hz ?
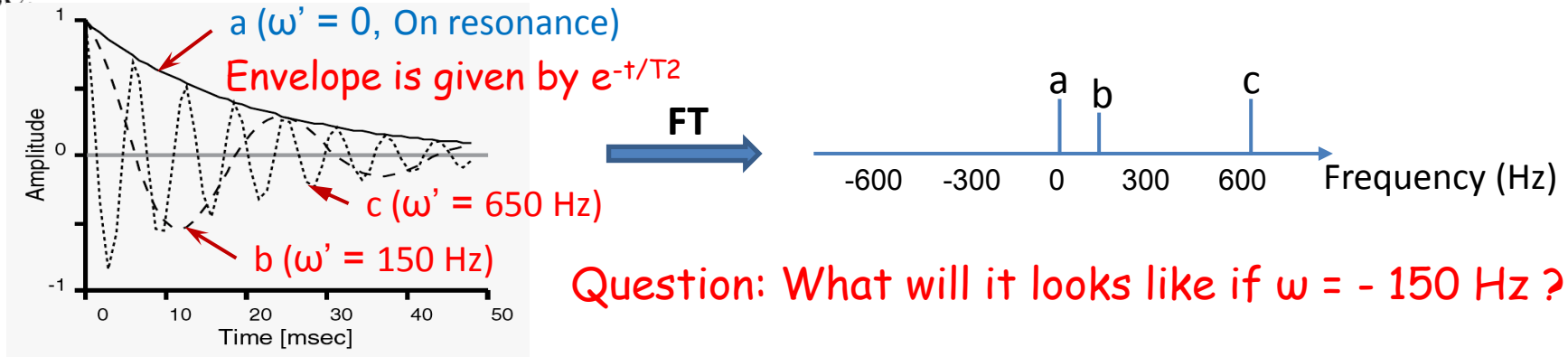
Quadrature detection (Detect the signal in both X- and Y-direction):
Signal at time t after the B1 pulse ends: ($\omega' = \omega_s - \omega$)

$$M_x(t) = M_o cos(\omega' t)e^{-t/T_2} \qquad M_y(t) = M_o sin(\omega' t)e^{-t/T_2}$$

$\omega' = \omega_s - \omega$ is the rotating frame resonance frequency
ω_s : The freq of RF pulse, i.e. coordinate rotating frequency.
ω : The actual resonance frequency in laboratory frame

These two signals are usually combined into a single complex number:

$$S(t) = M_x(t) + iM_y(t) = M_o e^{i\omega' t}e^{-t/T_2}$$
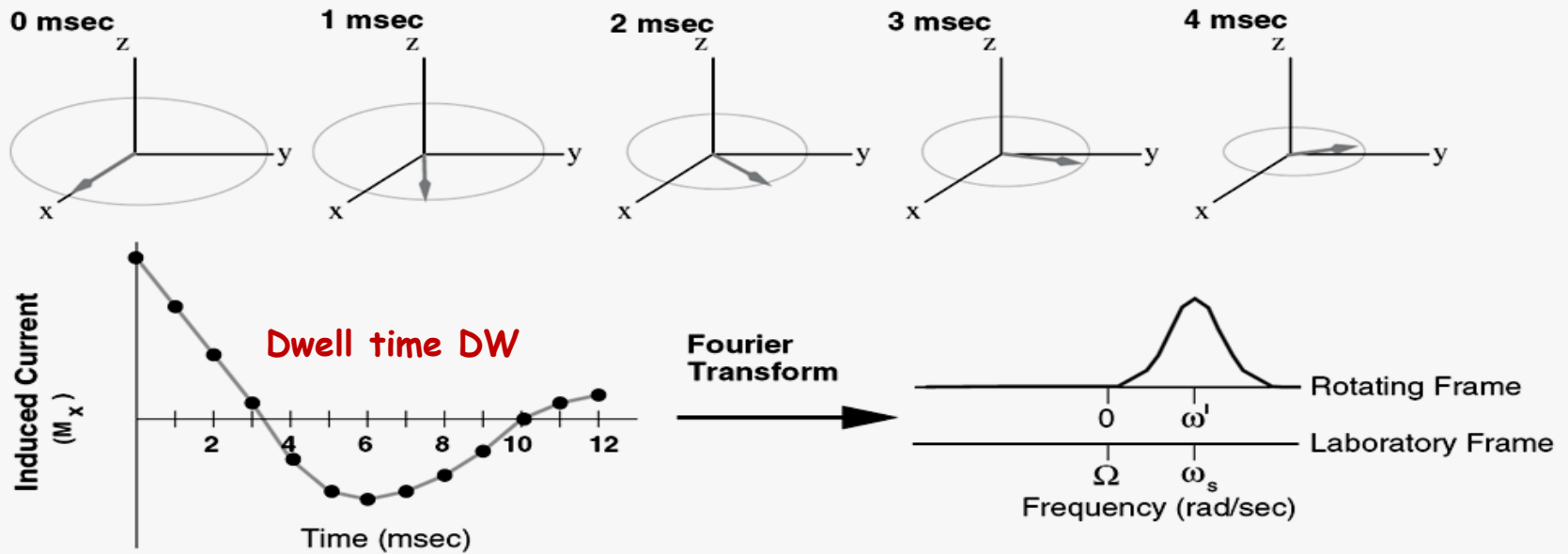
Figure 1.11. *Fourier transform of the time domain signal.* The free induction decay after the 90° pulse is shown. The upper section of the figure shows the precession of the transverse (i.e. x-y) magnetization after the pulse. The lower part of the figure shows the FID with the points indicating the data sampled during digitization, representing a dwell time of 1 msec. The subsequent resonance line obtained after Fourier transformation is shown to the right. In this case the pulse is slightly off-resonance and precesses in the rotating frame. The upper scale for the abscissa of the spectra gives frequencies in the rotating frame, the lower scale gives frequencies in the laboratory frame.

Question: What are the FT of M_x, M_y, S(t) and $e^{-t/T2}$?

FT of $e^{-t/T2}$ is: $F(\omega) = \dfrac{T_2}{1 + T_2^2 \omega^2}$ Lorentzian lineshape

This lineshape is shown in Fig. 1.12. The full width of the line at half-height, $\Delta\nu$, is inversely proportional to the T_2: $\Delta\nu = 1/(\pi T_2)$.
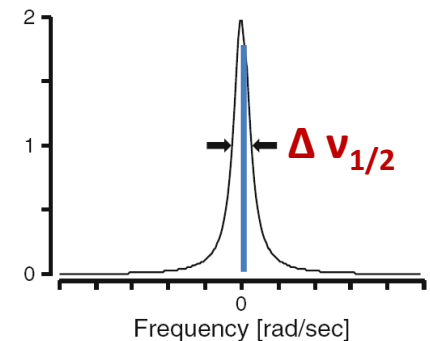
FT($e^{i\omega t}$) = δ(ω) (A delta function at ω)   FT($e^{-t/T2}$) = $\dfrac{T_2}{1+T_2^2\omega^2}$

FT[$e^{-i\omega t}$ · $e^{-t/T2}$] = δ(ω) ⊗ $\dfrac{T_2}{1+T_2^2\omega^2}$
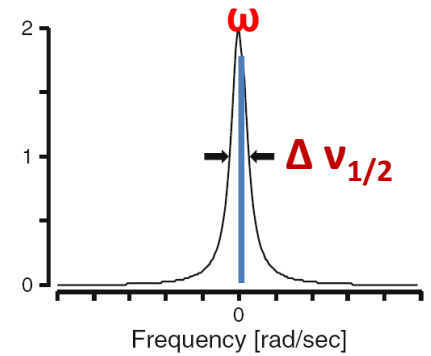
1.4   Phenomenological Description of Relaxation

First order relaxation process: $I(t) = I_o e^{-t/T} = I_o e^{-Rt}$

T is the relaxation time (sec) and R is the relaxation rate (sec^{-1}) and R = 1/T.

T_1: Spin-lattice (Longitudinal) relaxation (Relaxation in Z-direction) (Energy dissipation).

T_2: Spin-spin (Transverse) relaxation (relaxation on x-y plane)
   (Dephasing or lost of coherent).
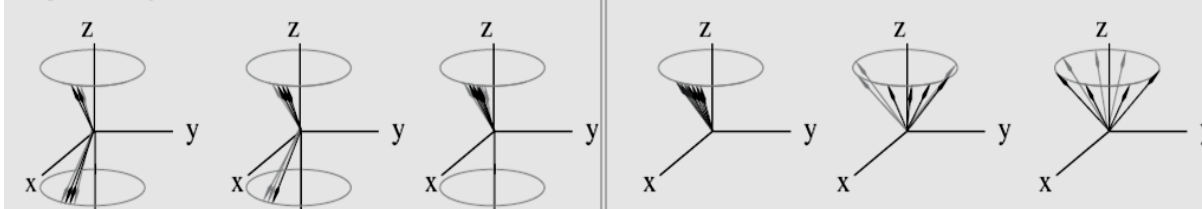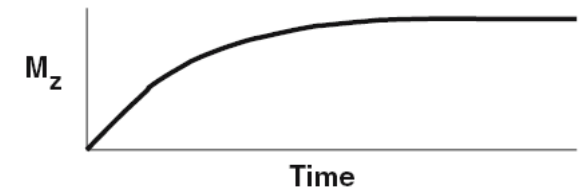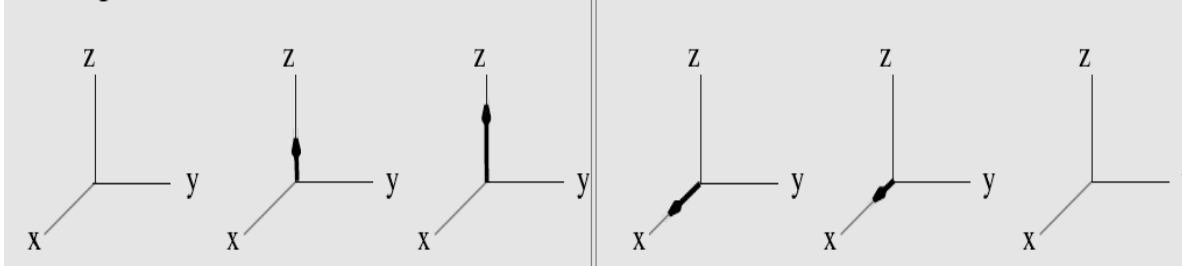
Factors affecting T_2:

1. Field inhomogeneity: Cause spins at different location to resonate at different frequency.
2. Dipolar coupling between adjacent spins (spin-spin coupling). Causes splitting.
3. Chemical shield anisotropy (CSA) due to anisotropic distribution of electrons around the nucleus.

Thus,
$$R_2^* = \frac{1}{T_2^*} = R_2^{\Delta B} + R_2^{DD} + R_2^{CSA}$$

In cases where $R_2^{\Delta B}$ can be ignored:
$$\frac{1}{T_2} = R_2 = R_2^{DD} + R_2^{CSA}$$

1.4.1 Relaxation and the Evolution of Magnetization

$$\frac{d\vec{M}}{dt} = \gamma \vec{M} \times B$$

The decay of magnetization :
(Phenomenological description)
The decay terms are added to
account for relaxation.

$$\frac{dM_z}{dt} = \frac{M_o - M_z}{T_1} + \gamma(M \times B)_z$$
$$\frac{dM_x}{dt} = \frac{-M_x}{T_2} + \gamma(M \times B)_x$$
$$\frac{dM_y}{dt} = \frac{-M_y}{T_2} + \gamma(M \times B)_y$$

(Bloch equations)

In the rotating frame:
$$\frac{\delta M_z}{\delta t} = \frac{M_o - M_z}{T_1}$$
$$\frac{\delta M_x}{\delta t} = \frac{-M_x}{T_2} + M_y(\omega_s - \omega)$$
$$\frac{\delta M_y}{\delta t} = \frac{-M_y}{T_2} - M_x(\omega_s - \omega)$$

where $\omega_s = \gamma B, \omega = -\Omega$.

Let $\quad M^+ = M_x + iM_y \quad$ then

$$\frac{\delta M^+}{\delta t} = -M^+ \left[\frac{1}{T_2} + i\omega'\right] \qquad (1.42)$$

The solution to eq. (1.42) is:

$$M^+ = e^{-i\omega't}\, e^{-t/T_2} \qquad (1.43)$$

The magnetization after a $P_{90}{}^x$ pulse we have the intitial conditions: $M_x = M_z = 0$ and

$$M_x(t) = \sin(\omega't)\, e^{-t/T_2}$$
$$M_y(t) = \cos(\omega't)\, e^{-t/T_2}$$
$$M_z(t) = M_o[1 - e^{-t/T_1}]$$
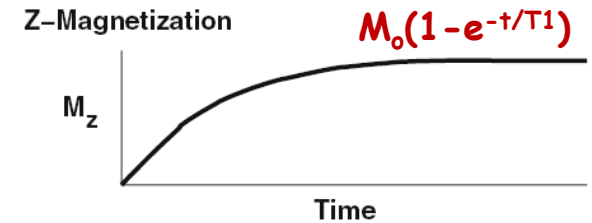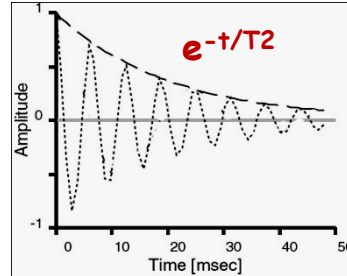
In quadrature detection the signal:

$$\underset{\text{Real}\qquad\text{Imaginary}}{S(t) = M_x(t) + iM_y(t) = M_o e^{i\omega't} e^{-t/T_2}} \qquad (1.31)$$

1.5 Chemical Shielding $\qquad B = (1 - \sigma)\cdot B_o$

Lamb formula: For isotropic electron distribution: σ is call chemical shift.

$$\sigma = \frac{e^2}{3mc^2}\int \frac{\rho(r)}{r}\,dr$$

For an anisotropic distribution σ is a tensorial quantity:

$$\sigma = \begin{bmatrix} \sigma_{xx} & 0 & 0 \\ 0 & \sigma_{yy} & 0 \\ 0 & 0 & \sigma_{zz} \end{bmatrix}$$

In solid the resonance frequency of a spin depends on its orientation w.r.t. the magnetic field and equals σ_{xx} if along the x-direction and σ_{yy} if along Y-direct and σzz is along the Z-direction.

In solution, it averages to a scale quantity:

$$\bar{\sigma} = \frac{1}{3}\left[\sigma_{xx} + \sigma_{yy} + \sigma_{zz}\right]$$

σ is proportional to B but if we define $\delta = \dfrac{\nu - \nu_o}{\nu_o} \times 10^6$ then the chemical shift will be independent of the field the spectrum is taken. Here ν_o is the frequency of the RF pulse and ν is the resonance frequency of the spin. σ has the unit of ppm (part per million). This makes it possible to directly compare the position of resonance lines in spectra obtained at different field.

Example: If a spin resonates at 2 ppm then this spin will resonate at 600 Hz away from the reference frequency at a 300 MHz spectrometer (i.e. ^1H spin resonates at ~300 MHz). This spin will resonate at 1800 Hz away from the reference if the spectrum is taken at 900 MHz spectrometer.

In 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.
For ^1H the chemical shift of a functional group is usually scattered around a defined region given below:

	15	10	7	5	2	0	ppm
100 MHz :	1500	1000	700	500	200	0	(Hz)
300 MHz:	4500	3000	2100	1500	600	0	(Hz)
500 MHz:	7500	5000	3500	2500	1000	0	(Hz)
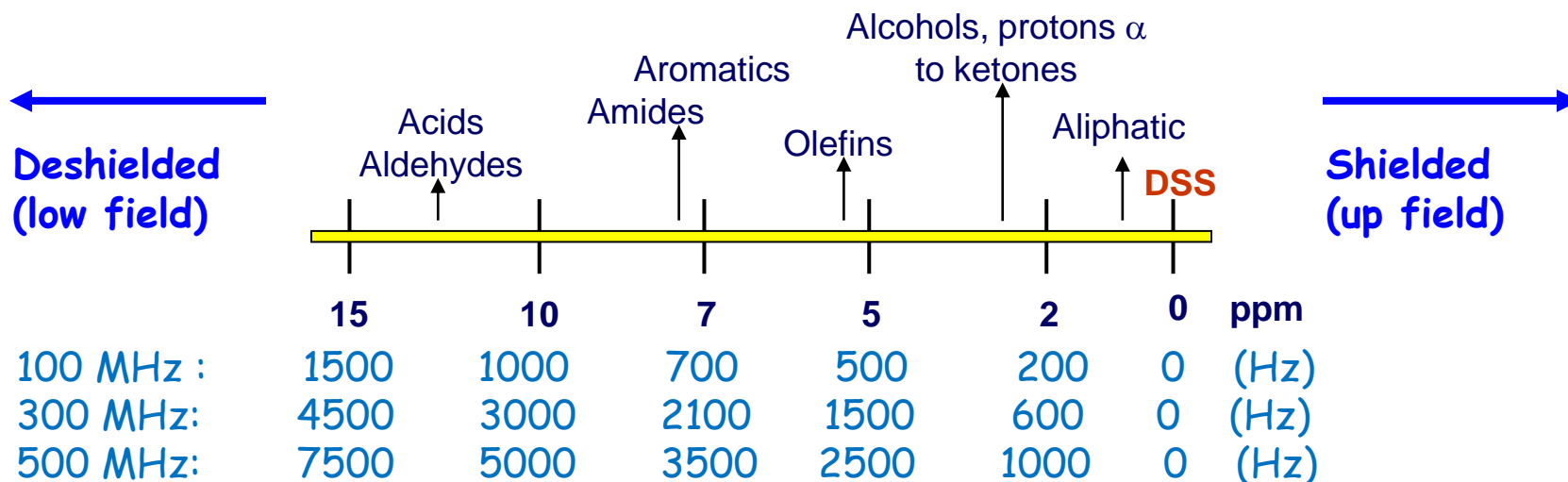
Chemical Shift Referencing: The ^{1}H chemical shift was referenced to 2,2-dimethyl-2-Silapentane-5-sulfonate (DSS) at 0 ppm. The ^{15}N and ^{13}C chemical shift values were referenced using the consensus ratio of Ξ of 0.101329118 and 0.251449530 for ^{15}N/^{1}H and ^{13}C/^{1}H, respectively
    (Wishart and Case, Method. Enzymol. 338, 3-34 (2001))

TABLE 1
### IUPAC/IUBMB RECOMMENDED Ξ (XI) RATIOS FOR INDIRECT CHEMICAL SHIFT REFERENCING IN BIOMOLECULAR NMR[a]

Nucleus	Compound	Ξ Ratio
^{1}H	DSS	1.000 000 000
^{13}C	DSS	0.251 449 530
^{15}N	Liquid NH_3	0.101 329 118
^{19}F	CF_3COOH	0.940 867 196
^{31}P	$(CH_3)_3PO_4$	0.404 808 636

[a] Relative to DSS.

Ξ ratio (Nucleus-specific frequency ratio: Determine the precise ^{1}H resonance frequency of DSS then multiply this frequency by Ξ of a particular nucleus one obtains the exact resonance frequency reference at 0 ppm of that nucleus.

胺基酸為生物蛋白質的基本組成單位。雖然蛋白質所含的胺
基酸數目十分龐大，但是這些蛋白質胺基酸多半是由同樣的
二十種不同的胺基酸所重複排列組合而成。這些胺基酸的共
同結構如下（R為Remainder，代表該分子其他剩餘的部分）

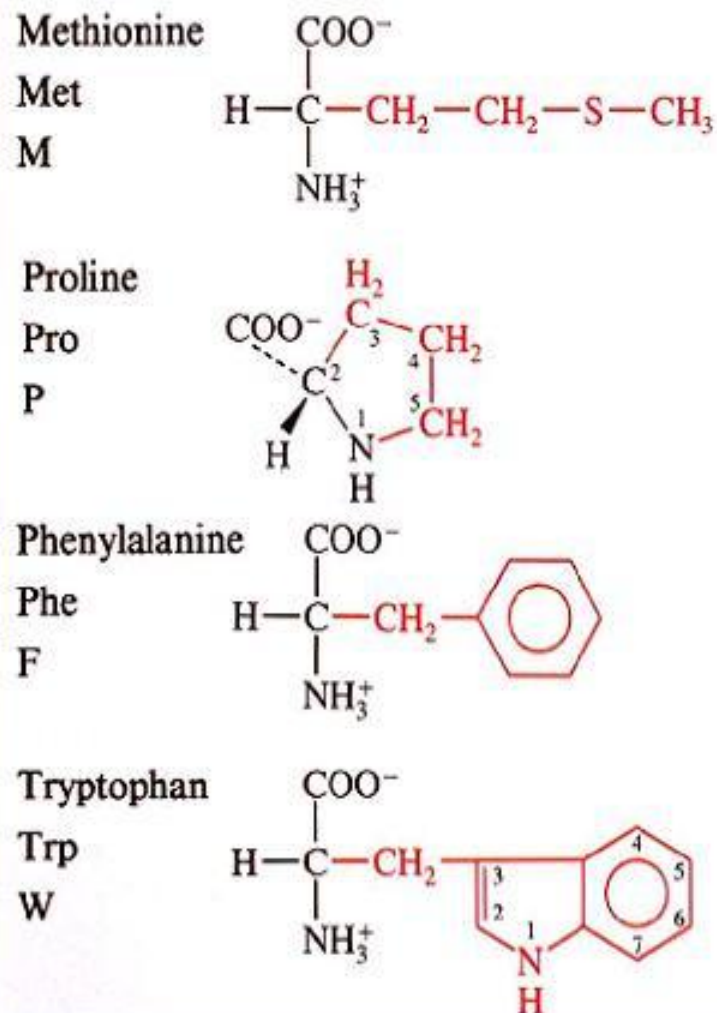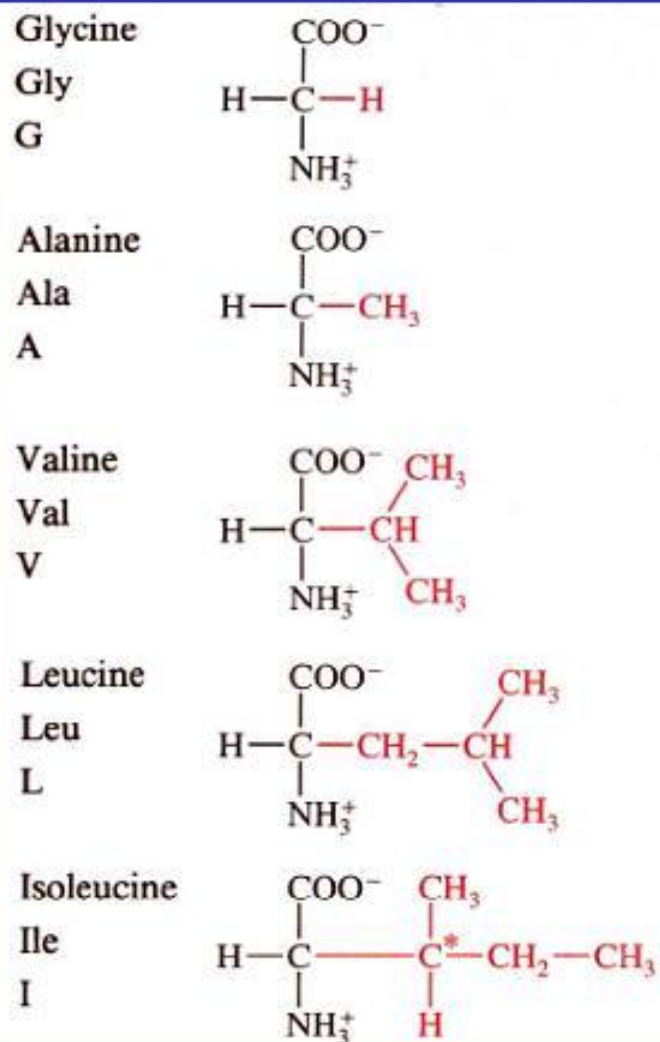$$H_2N-C_\alpha-COOH$$

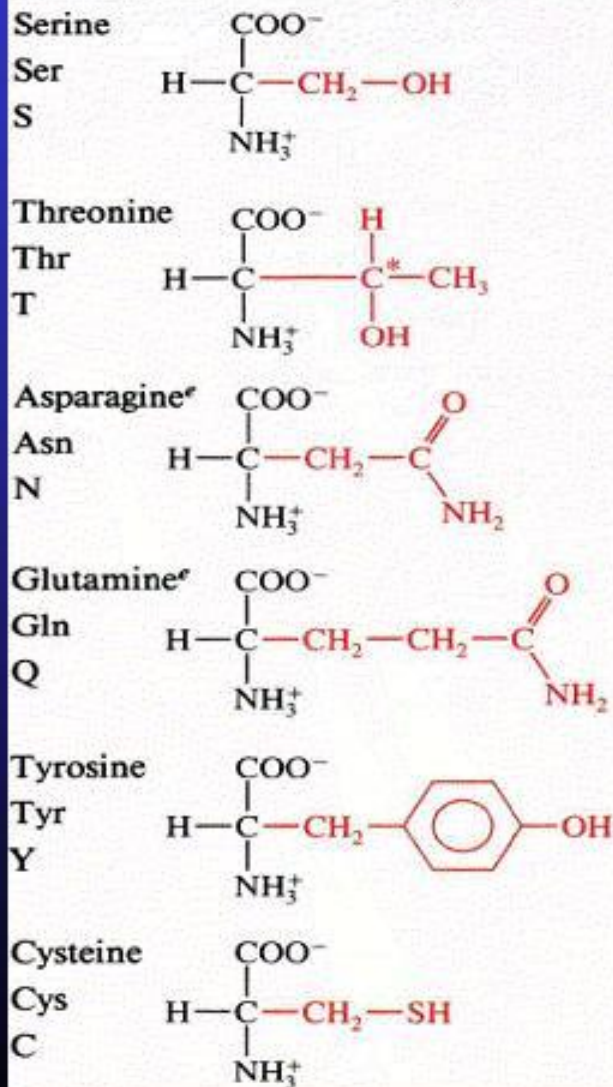$$H_3\overset{+}{N}-C-COO^-$$
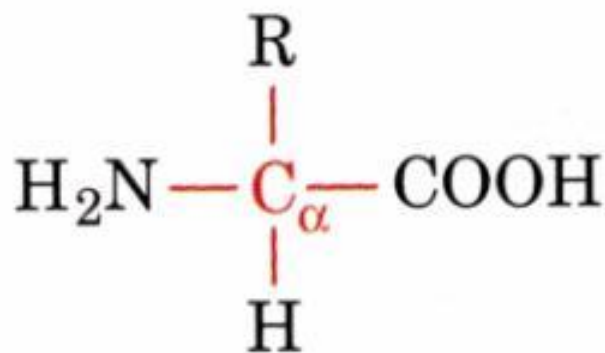
胺基酸的通式結構
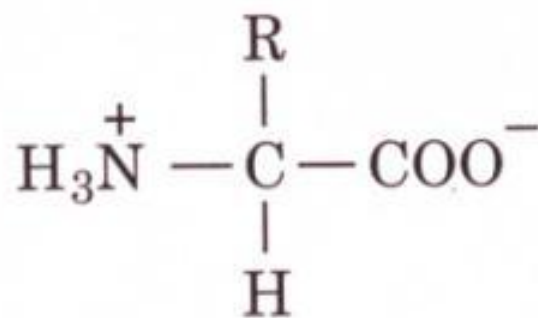
胺基酸的極性結構（zwitterionic
form通常發生於生理狀態pH值時）

這常見於細胞中二十種不同的胺基酸的分類可依其分支分子（R部分）的極性來劃分。其中非極性分支端的胺基酸有九種，其分支端的大小與幾何形狀各異。

十一種極性分支端的胺基酸，又可分為六種為帶電極性分支端胺基酸與五種可能帶電極性分支端胺基酸。
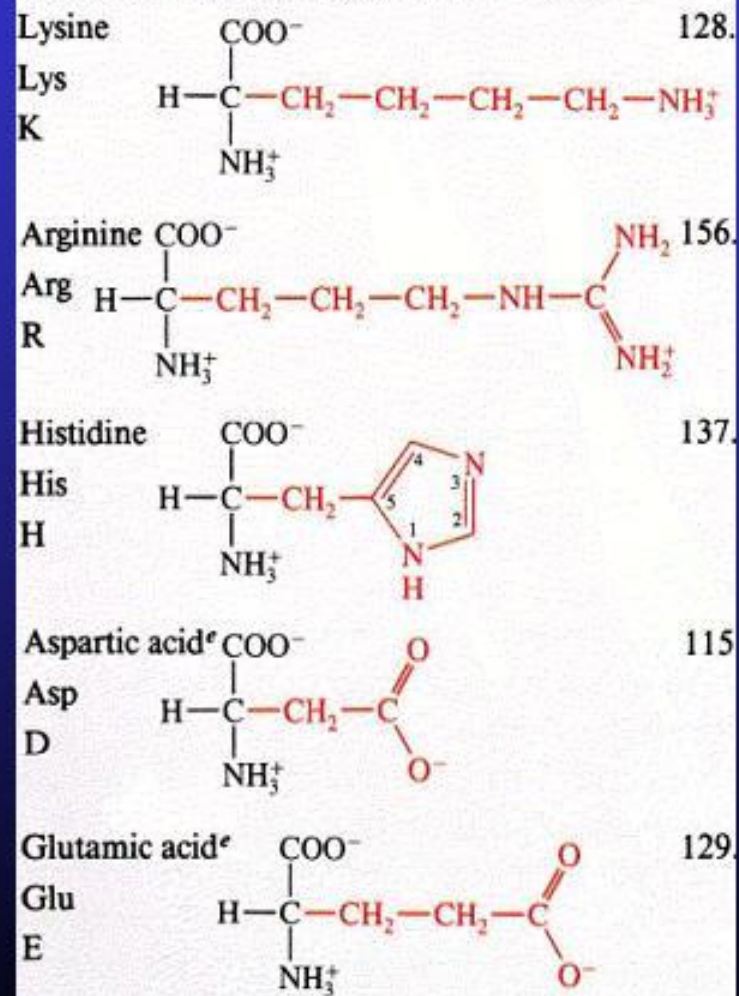

Amino acids with uncharged polar side chains

Serine Ser S

Threonine Thr T

Asparagine[e] Asn N

Glutamine[e] Gln Q

Tyrosine Tyr Y

Cysteine Cys C

Amino acids with charged polar side chains

Lysine Lys K 128.

Arginine Arg R 156.

Histidine His H 137.

Aspartic acid[e] Asp D 115

Glutamic acid[e] Glu E 129.

Table 1.2. *Proton chemical shifts.* The average proton chemical shifts in proteins are shown. These data were obtained from BioMagResBank [52].

Residue	NH	H_α	H_β	Others
Gly	8.34	3.94		
Ala	8.20	4.26	1.38	
Val	8.29	4.16	1.99	0.84, 0.83(CH3)
Ile	8.26	4.20	1.80	1.30, 1.24 (CH2), 0.80 (γCH3), 0.70 (δCH3)
Leu	8.22	4.32	1.63,1.57	1.54 (γCH), 0.77, 0.76(δCH3)
Pro	-	4.41	2.05,2.05	1.93 (γCH2), 3.64, 3.63 (δCH2)
Ser	8.29	4.51	3.88	5.33 Hγ (OH)
Thr	8.27	4.48	4.17	1.16 (γCH3), 4.40 Hγ1 (OH)
Asp	8.33	4.61	2.74,2.70	
Glu	8.34	4.26	2.04	2.31 (γCH2)
Lys	8.22	4.28	1.79,1.78	1.38 (γCH2), 1.61 (δCH2), 2.93 (ϵCH2), 7.52 (ζNH3)
Arg	8.24	4.27	1.79	1.58 (γCH2), 3.13 (δCH2), 7.32, 6.74, 6.72 (NH)
Asn	8.37	4.70	2.80,2.78	7.27, 7.20 (δNH2)
Gln	8.22	4.28	2.05,2.04	2.32 (γCH2), 7.17, 7.07 (γNH2)
Met	8.26	4.39	2.03,2.01	2.44 (γCH2), 1.86 (ϵCH3)
Cys	8.42	4.73	2.95,2.98	1.66 -SH
Trp	8.35	4.74	3.32,3.18	6.68-7.17 (aromatic), 10.13 (NH)
Phe	8.42	4.62	2.97,2.99	6.89-6.91 (aromatic)
Tyr	8.37	4.63	1.91	6.86 (Hδ), 6.64 (Hϵ), 9.25 (-OH)
His	8.25	4.62	3.11,3.12	Hδ1 10.14(NH), Hδ2 7.08, Hϵ1 8.08, Hϵ2 10.43(NH)

Note that within a residue, the relationship between atom type and chemical shift is similar for both carbon and proton shifts. For example, in the case of arginine the following ordering is found for both carbon and proton shifts: $\alpha > \delta > \beta > \gamma$ (see

Table 1.3. Nitrogen chemical shifts. The nitrogen chemical shifts for side-chain atoms are shown. The amide nitrogen chemical shifts are ≈ 120 ppm, with the exception of glycine, which is found at 109.9 ppm. Data from BioMagResBank [52].

Residue	Shifts	Residue	Shifts
Arg	89.8 (ϵ), 74.8 NH1, 75.8 NH2	His	190.7 (δ1), 179.8 (ϵ2)
Asn	112.8 (δ)	Lys	71.86 (ζ)
Gln	111.8 (ϵ)	Trp	129.5 (ϵ)

Table 1.4. Carbon chemical shifts. The average carbon chemical shifts were obtained from the BioMagResBank [52]. Carbonyl shifts have been omitted from this table since they are quite uniform at approximately 175 ppm.

Residue	C_α	C_β	Others
Gly	45.3		
Ala	53.1	18.9	
Val	62.5	32.6	21.3 (CH3)
Ile	61.6	38.6	27.6 (γ1), 17.3 (γCH3), 13.4 (δCH3)
Leu	55.7	42.3	26.8 (γ), 24.5 (δCH3)
Pro	63.3	31.8	27.1 (γ), 50.3 (δ)
Ser	58.6	63.8	
Thr	62.1	69.6	21.4 (γCH3)
Asp	54.5	40.7	178.41 (γ) sidechain
Glu	57.4	30.0	36.0 (γ), 181.9 (δ) sidechain
Lys	56.8	32.8	24.9 (γ), 28.8 (δ), 40 (ϵ)
Arg	56.9	30.7	27.3 (γ), 43.1 (δ), 159.0 (ζ)
Asn	54.5	40.7	178.41 (γ) sidechain
Gln	56.6	29.1	33.7 (γ), 179.7 (δ) sidechain
Met	56.1	32.9	32.1 (γ), 17.2 (ϵCH3)
Cys	57.4	34.1	
Trp	57.7	30.1	110-137 (aromatic)
Phe	58.2	40.0	129-138 (aromatic)
Tyr	58.0	39.1	117 (ϵC), 132 (δC), 156 (ζ)
His	56.4	30.0	119.8 (δ2), 136 (ϵ1)

Figure 1.16. Distribution of carbon and proton chemical shifts. The distribution of observed carbon (A, left) and proton (B, right) chemical shifts in proteins. The solid circles (·) mark the average chemical shift. The solid lines indicate ±3σ; 95% of the observed chemical shifts fall within this range. The gray boxes indicate nominal chemical shift ranges for α, β, and methyl atoms. In the case of carbon shifts, these ranges separate the atom types quite well. Note that there are a few exceptions, for example, the β-carbons of Ser and Thr fall in the α-region and the α-carbon of Gly can fall in the β-carbon region. The large range of β-carbon shifts for Cys is due to the fact that both free and disulfide bonded residues are included in this figure. In the case of proton shifts, the separation by atom type is not as clean due to the extensive chemical shift overlap between the various atom types. Data from the BioMagResBank database of chemical shifts [52].
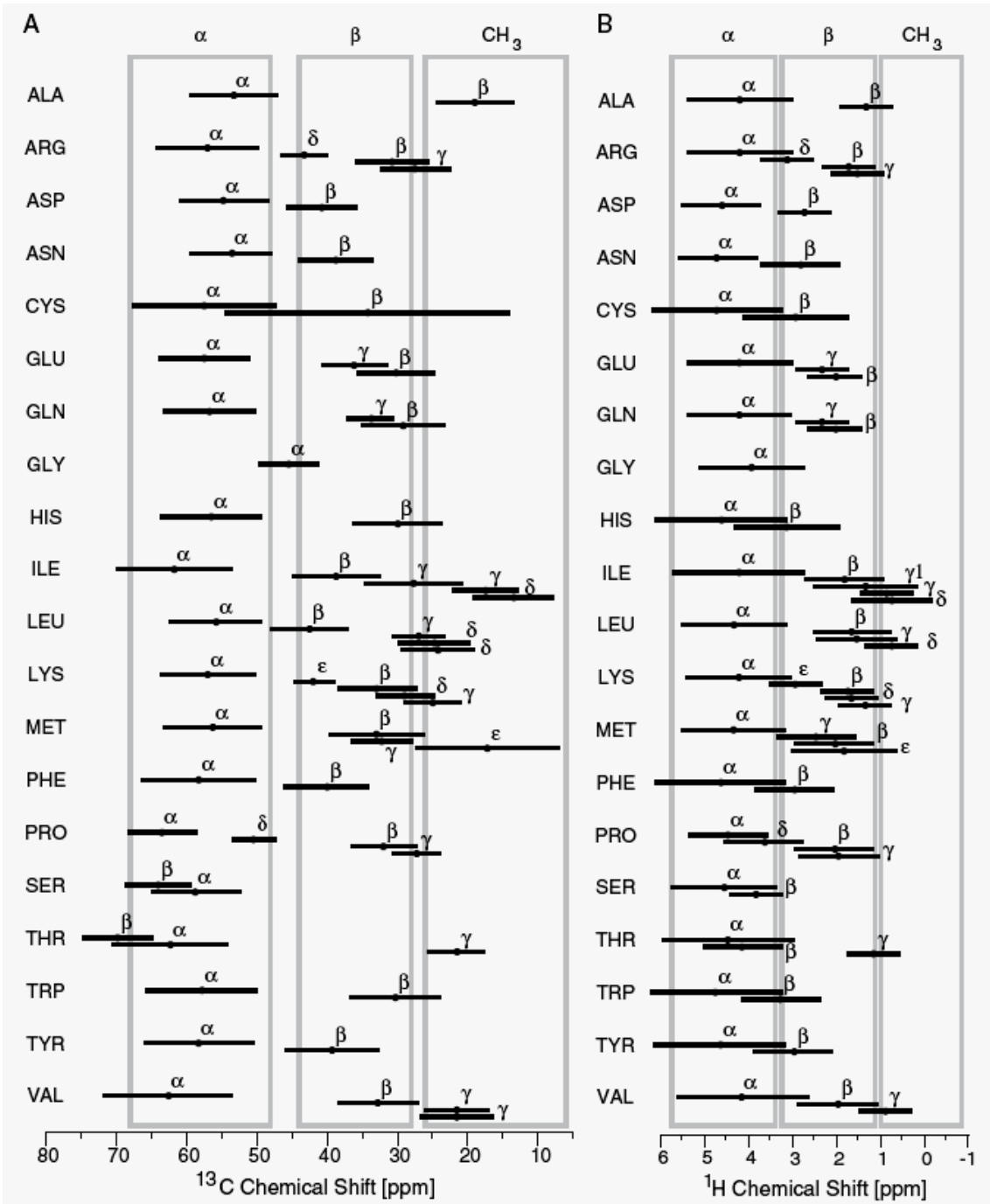
Nitrogen (400 ppm) and carbon (200 ppm) have much larger range of chemical shifts.

In addition to the chemical bonding effect chemical shift is also affected by many external factors, such as: (1) Secondary structure; (2) Hydrogen bonds; (3) Charge near the spin. Positive charge withdraw electrons from the spin and causes de-shielding (Larger chemical shift) and positive charge has the opposite effect. (4) Ring current shift ; (5)Electron spins (paramagnetic shift) etc.

1.6.2   Ring Current Effects

Aromatic groups have delocalized electrons that circulating around the ring and behaves like a coil to generate magnetic fields which affect the chemical shift of adjacent spins.

The dipolar field is given by:

$$\sigma = iB\frac{1 - 3cos^2\theta}{r^3}$$
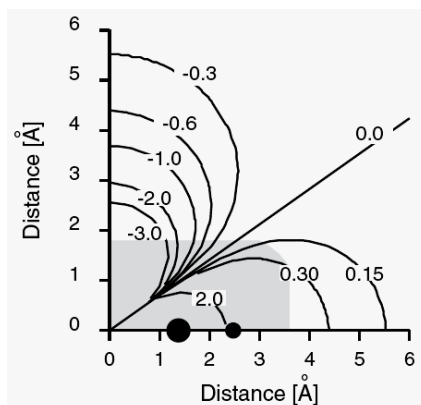

Figure 1.15   Ring current shifts. Calculated ring current shifts for a phenylalanine ring. The x-axis lies in the plane of the ring and the y-axis is perpendicular to the plane of the ring. The location of the carbon and its attached hydrogen are indicated by the large and small spheres, respectively. The large gray area represents the approximate Van der Waals radius of the phenyl group. The lines represent contours of iso-chemical shift changes.

1.6.3.1   Degeneracy and Equivalent Chemical shifts

1.6.4   Use of Chemical Shifts in Resonance Assignments

1.6.5 Chemical Shift Dispersion & Multi-dimensional NMR: Resolving the complex spectrum.

1.7. Exercises