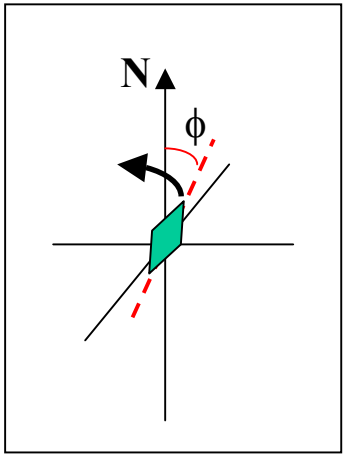
Conformational constraints from NMR experiments

Basic features

Because of a quantum-mechanical property named spin, the nucleus and the electrons of an atom can be regarded as charged particles in motion.

Therefore nucleus and electrons exhibit a magnetic dipole moment.

Basic features



Magnetic dipoles align to external magnetic fields, like the compass needle points towards the Earth magnetic North

Basic features

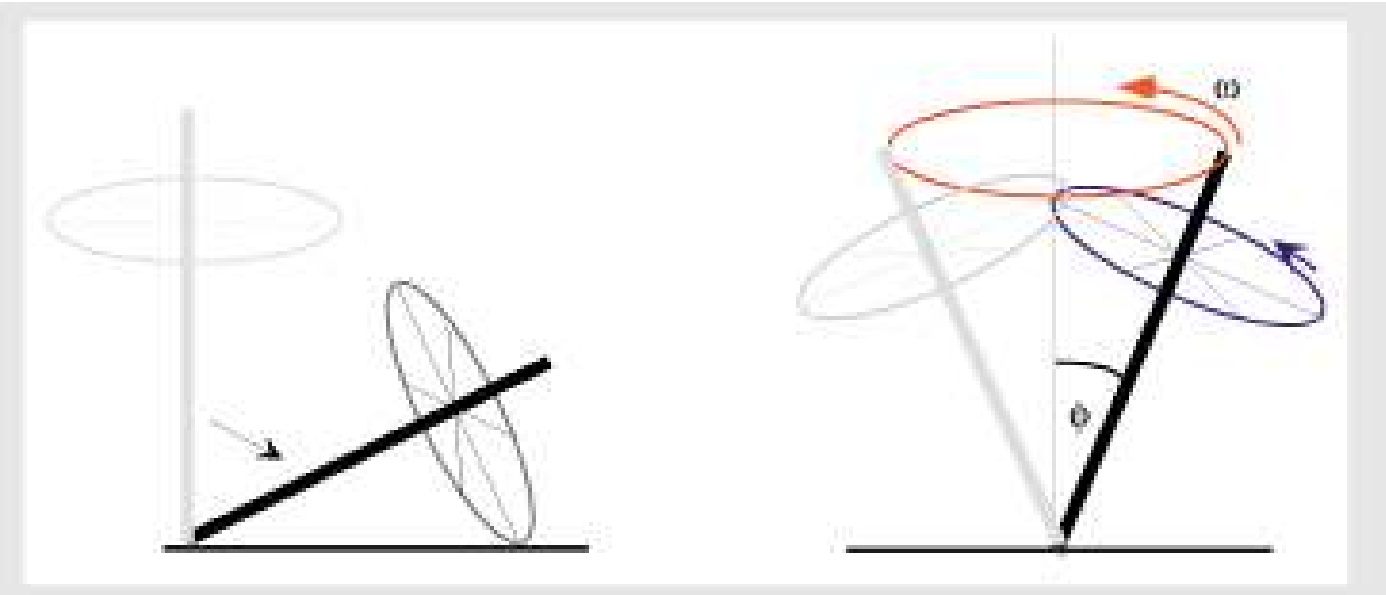
Nucleons, i.e. protons and neutrons, possess a spin that, like a rotation, entails a spin angular momentum, a quantum mechanical property that assumes only discrete values. The sum of the spin angular momenta of the individual nucleons builds up the overall nuclear spin angular momentum, that is still quantized.

The overall spin angular momentum of a nucleus, the currents of the protons and the intrinsic magnetism of the nucleus constituents are the basis of the nuclear magnetism that means a quantized nuclear magnetic moment.

A nuclear magnetic dipole in a magnetic field behaves like a gyroscope with an angular momentum that rotates around its axis and around the gravitational field axis (*precession motion*).

Basic features

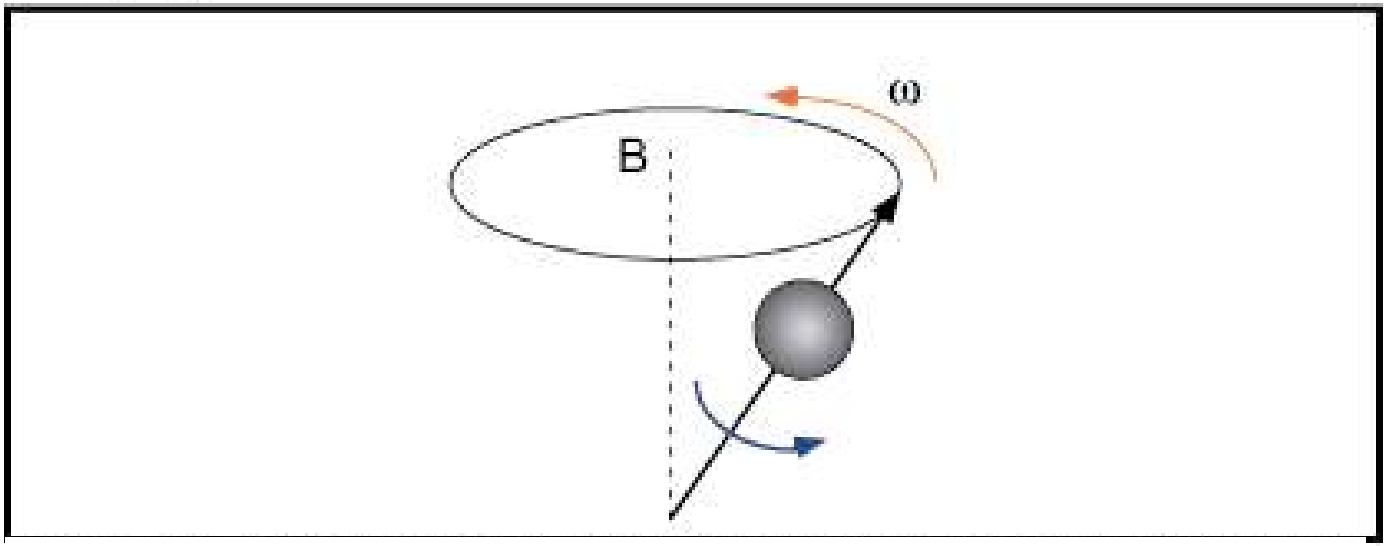
A gyroscope would fall down if left with its axis perpendicular to the ground.



By imparting a rotation, i.e. an angular momentum, under steady conditions (neglecting energy loss) the gyroscope *precesses* around the gravitational field direction, at a *precession* frequency (ω_p) that depends on the angular momentum (J):

$$\omega_p = \frac{mgr}{J}$$

Basic features



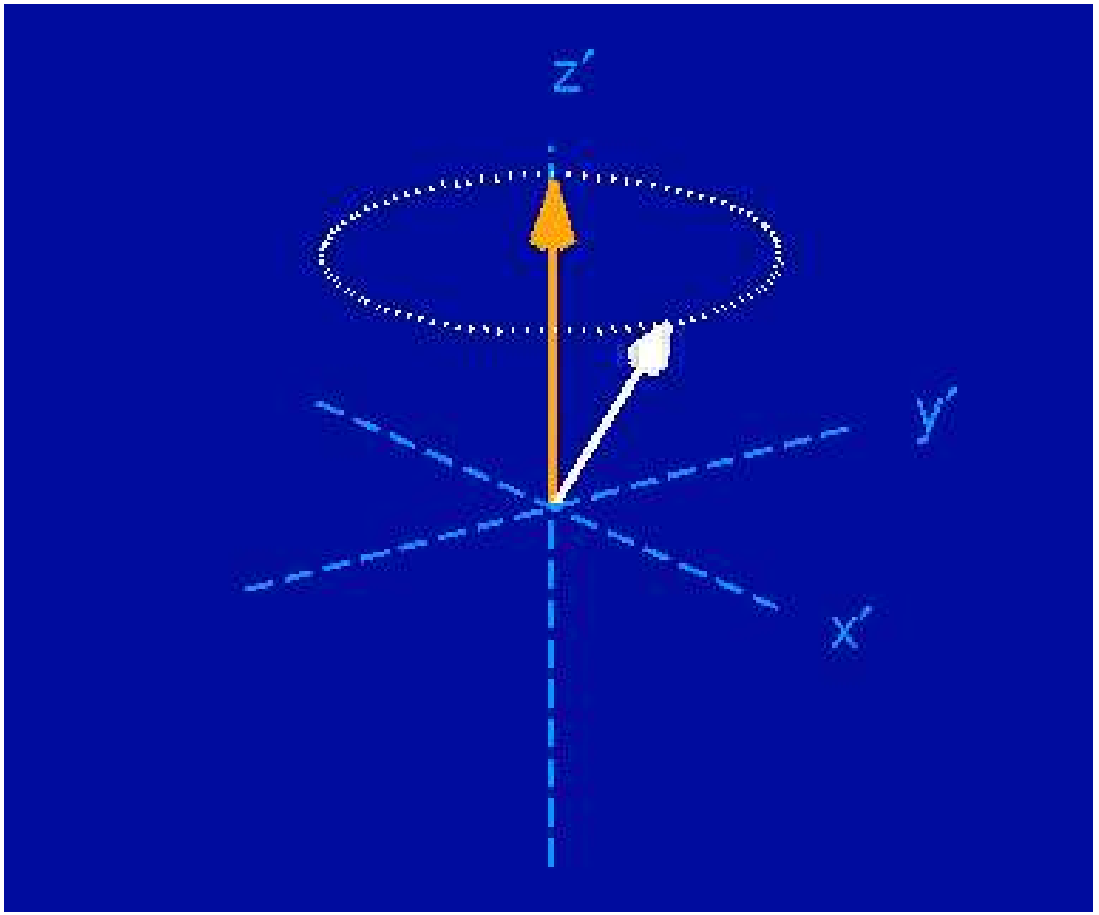
A nuclear magnetic dipole moment precesses around an external magnetic field axis according to:

$$\omega = -\gamma \cdot \mathrm{B} \quad \text{Larmor Equation}$$

$$\omega = 2\pi\nu \quad \Rightarrow \quad \nu = \frac{\gamma}{2\pi} B$$

at an angular frequency, ω, referred to as *Larmor frequency*.

Basic features

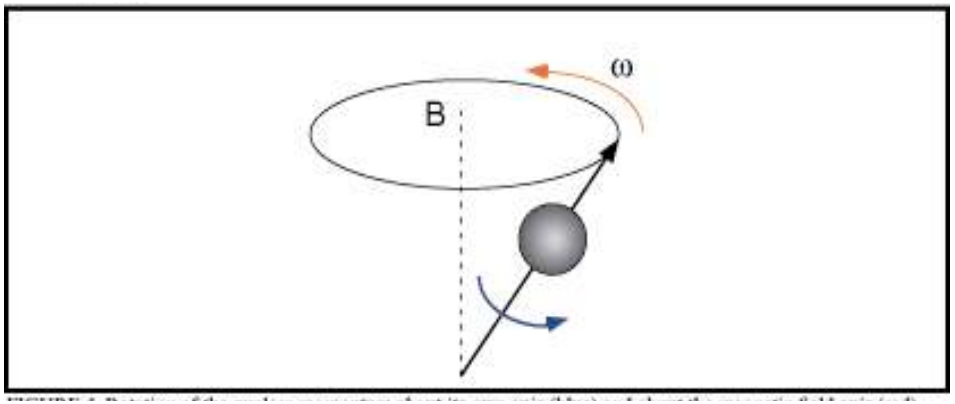


$$\omega = -\gamma \cdot \mathrm{B} \quad \text{Larmor Equation}$$

$$\omega = 2\pi\nu \quad \Rightarrow \quad \nu = \frac{\gamma}{2\pi} B$$

Movie: *Brian Hargreaves*

Basic features



$$\omega = -\gamma \cdot \mathrm{B} \quad \text{Larmor Equation}$$

$$\omega = 2\pi\nu \quad \Rightarrow \quad \nu = \frac{\gamma}{2\pi} B$$

The proportionality constant γ is named *gyromagnetic ratio* and represents the ratio between the magnetic moment and the angular momentum:

$$\gamma = \frac{\mu}{\mathrm{J}}$$

Analogy:

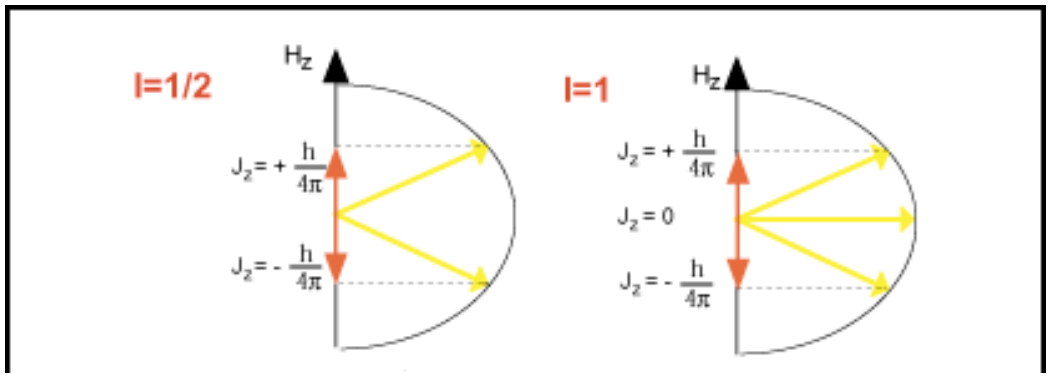
$$\omega = \gamma \cdot B = \frac{\mu \cdot B}{J} \qquad \omega_p = \frac{mgr}{J}$$

moment

The moment of a force has the same units as an energy, but is a vector.

Basic features

The nuclear spin angular momentum can adopt only discrete values, i.e. it is quantized. Hence, also the nuclear magnetic dipole moment will be quantized.



$$J = \hbar\left[I\left(I+1\right)\right]^{\frac{1}{2}} \quad J_z = \hbar m_I \quad m_I = I, I-1, I-2, \ldots -I \quad \text{(totally } (2I+1) \text{ values)}$$

$$\mu = \gamma \cdot J = \gamma\hbar\left[I\left(I+1\right)\right]^{\frac{1}{2}} \qquad \mu_z = \gamma \cdot J_z = \gamma\hbar m_I$$

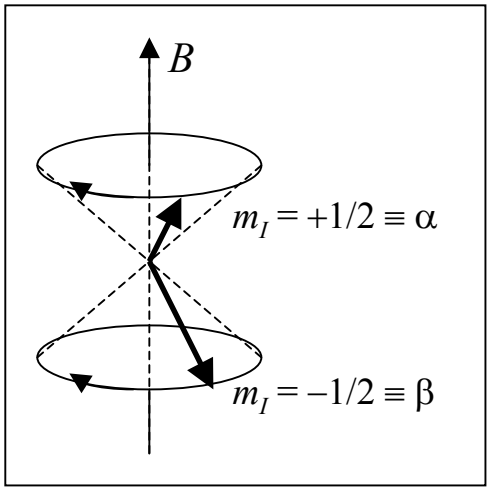
Nuclei with an odd mass number, have half-integer nuclear spin (^{1}H, ^{7}Li, ^{27}Al I= 1/2, 3/2 5/2).

Nuclei with even mass number and even atomic number, have zero nuclear spin (^{12}C, ^{16}O, ^{32}S).

Nuclei with even mass number and odd atomic number, have integer nuclear spin (^{2}H, ^{14}N, $I = 1$).

Basic features

$$\nu = \frac{\gamma}{2\pi} B \qquad \nu = \frac{|\omega|}{2\pi} \qquad \omega = -\gamma \cdot \mathrm{B} \qquad \text{Larmor Equation}$$



Larmor equation dictates the condition to observe nuclear magnetic resonance, i.e. applying the energy that is required to change the precession mode of the nuclear magnetization vector around the direction of a static magnetic field. For $I = 1/2$ there is a single possible transition $\alpha \rightarrow \beta$ or *vice versa*.

Larmor equation highlights the dependence of the resonance frequency on the magnetic field and the gyromagnetic ratio, a constant that is specific for every nucleus. For instance, at B = 2.3 T, the NMR signal from ^{1}H is observed at 100 MHz ($\gamma = 26.75 \cdot 10^{7}$ rad T^{-1} s^{-1}), from ^{13}C at 25.145 MHz ($\gamma = 6.73 \cdot 10^{7}$ rad T^{-1} s^{-1}), from ^{15}N at 10.14 MHz ($\gamma = -2.71 \cdot 10^{7}$ rad T^{-1} s^{-1}), from ^{31}P at 40.48MHz ($\gamma = 10.84 \cdot 10^{7}$ rad T^{-1} s^{-1}).

Basic features

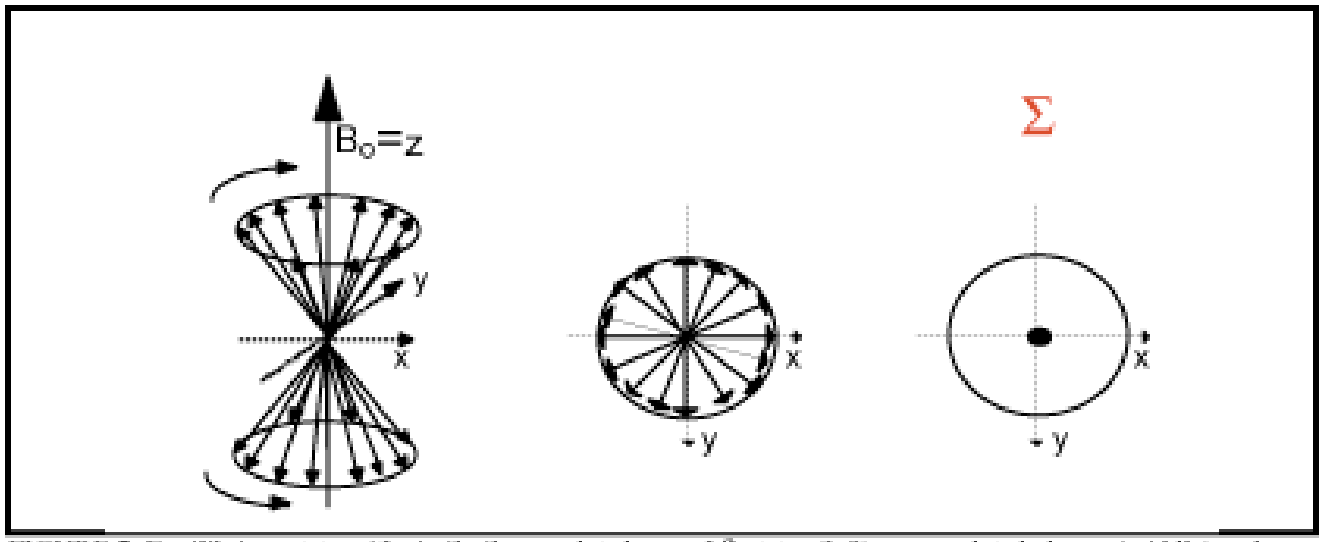


FIGURE 7. Equilibrium state with similarily populated α- and β-states (left), uncorrelated phases (middle) and no net phase coherence (right).

NMR deals with ensembles of nuclear magnetic moments, the nuclear spins, that sum up into a *macroscopic magnetization*.

Basic features

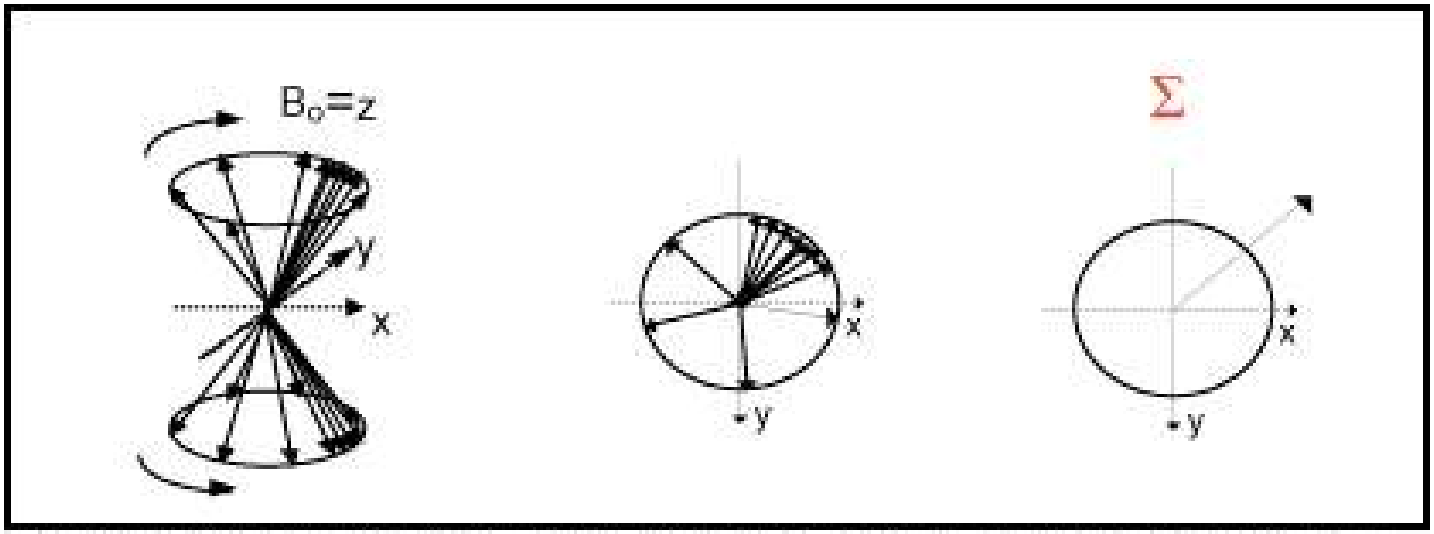


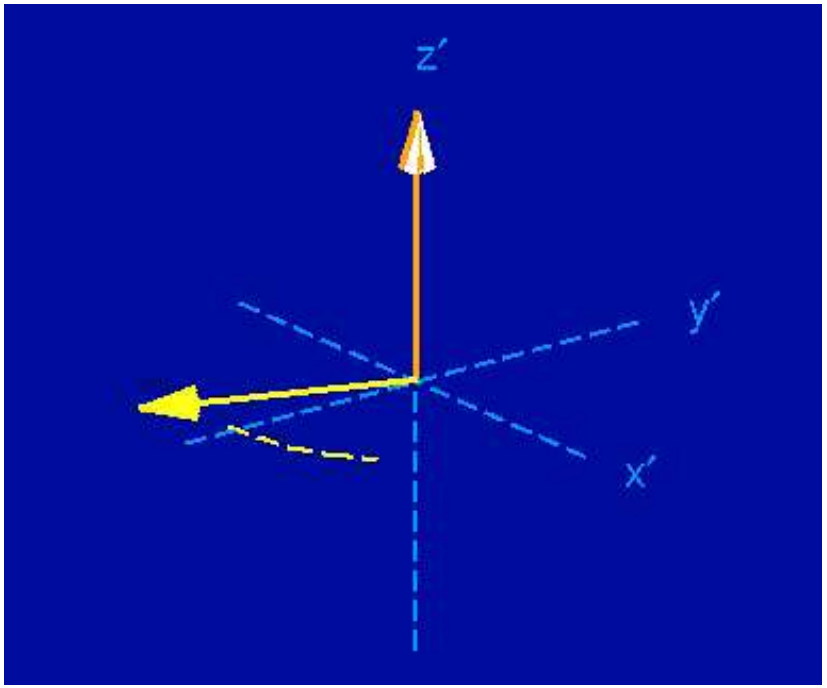
FIGURE 8. Coherent state of spins in α- or β(left) states and the projection onto the x,y plane(middle) and sum vector of the x,y component.

To observe a NMR signal, we must introduce some preferential distribution of the individual magnetization vectors, i.e. a *spin coherence*, that gives rise to a non-zero transverse magnetization.

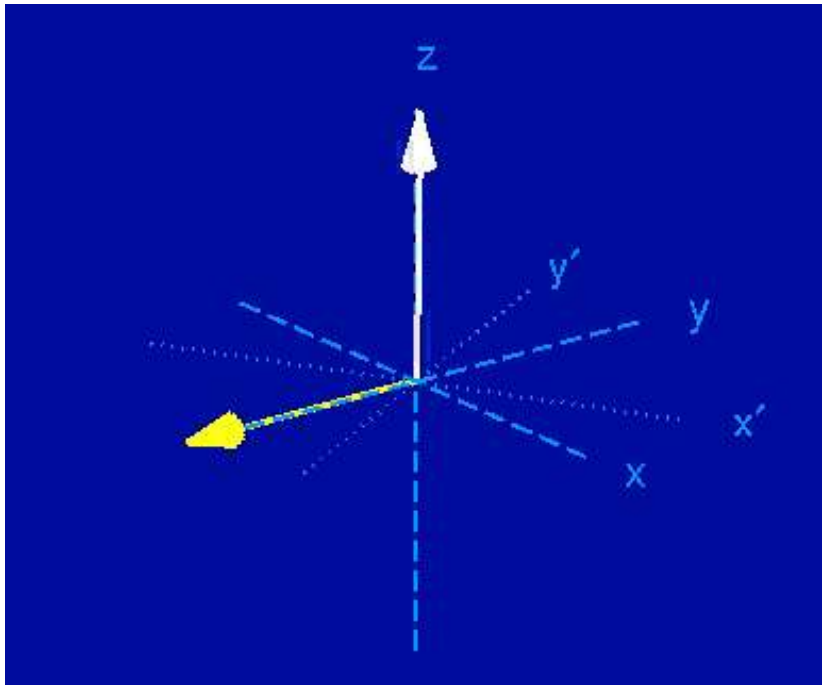
Only the transverse magnetization components are observed by NMR.

Basic features

The magnetic field B_1 associated to a radiofrequency oscillating at the Larmor frequency polarizes the macroscopic magnetization onto the x-y plane.



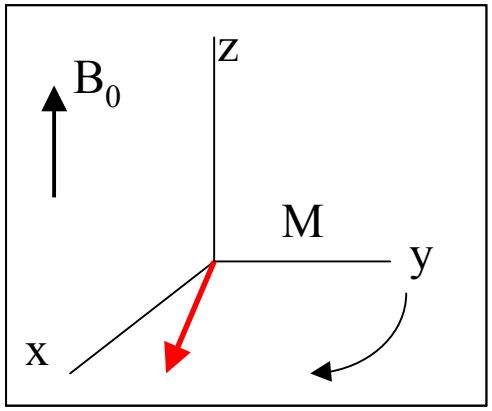
Laboratory frame



Rotating frame

Movie: *Brian Hargreaves*

Basic features



By the end of the radiofrequency pulse, in the absence of B_1, the tranverse magnetization starts to precess again around B_0.

Basic features

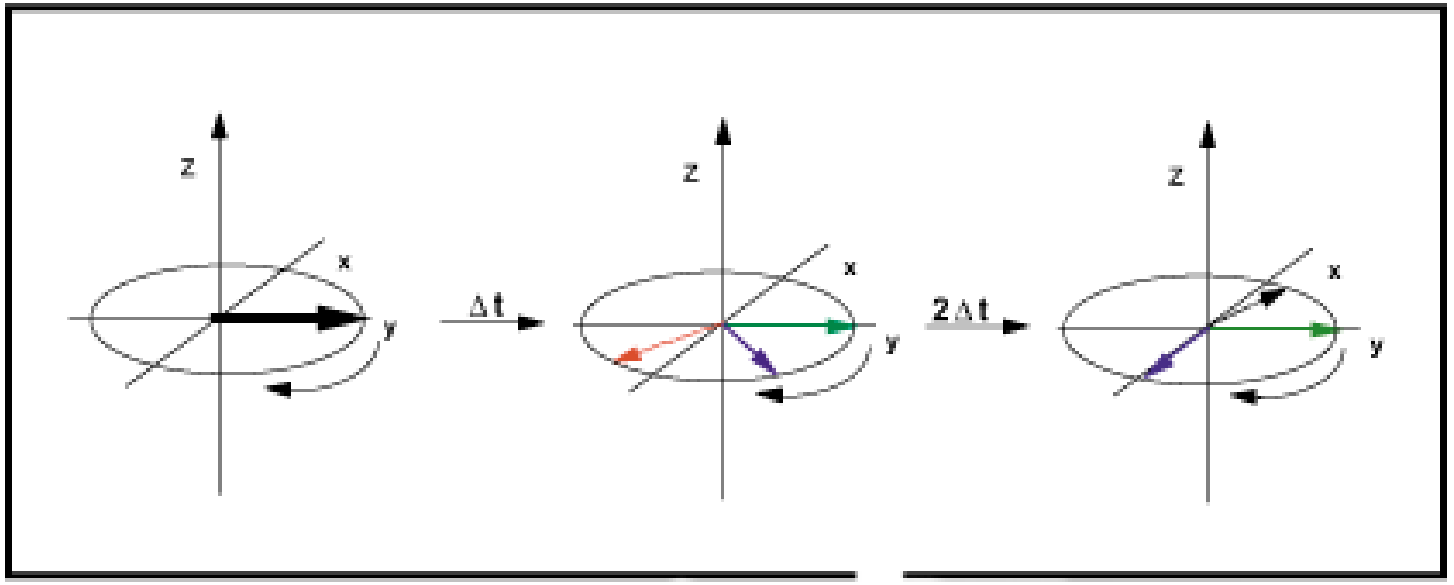
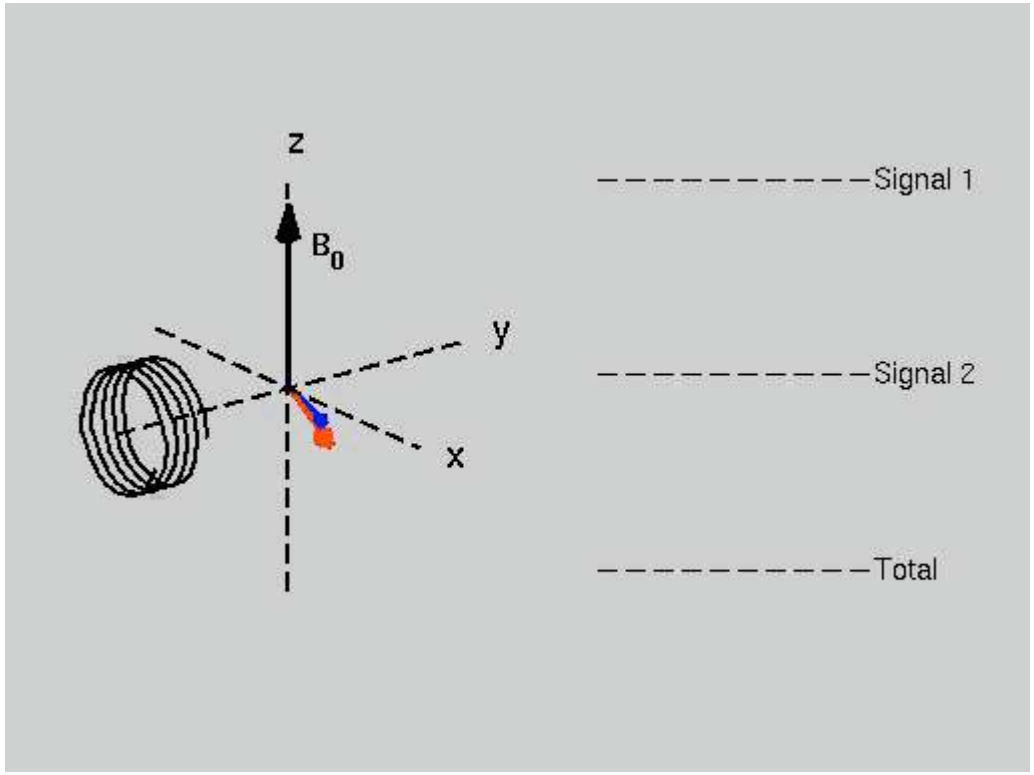


FIGURE 10. Spins precessing at different velocities

During precession, the transverse magnetization will split into components, if any, due to different Larmor frequencies.

Basic features



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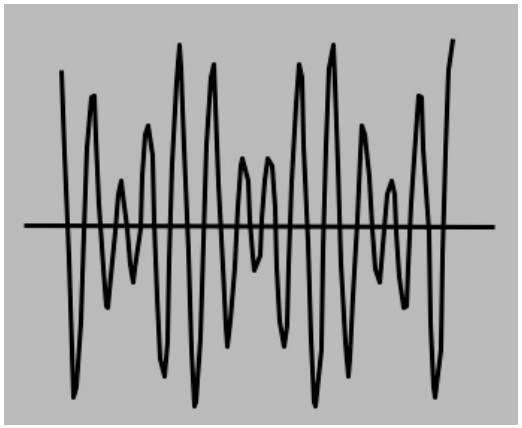
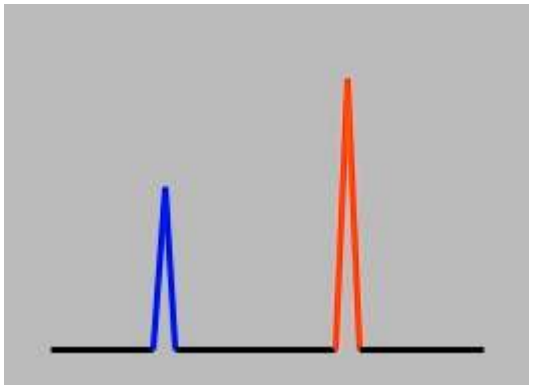


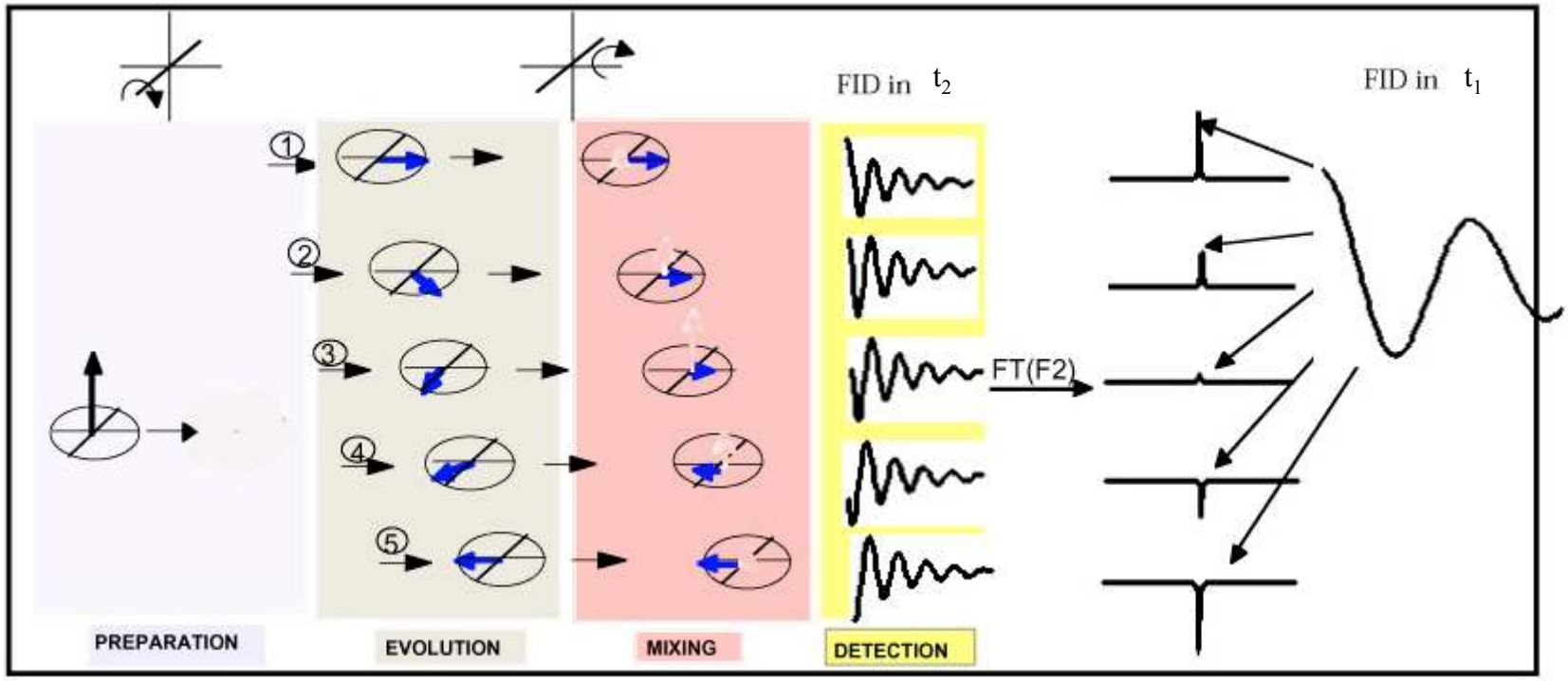
$$I(t) \xrightarrow{FT} I(\nu)$$



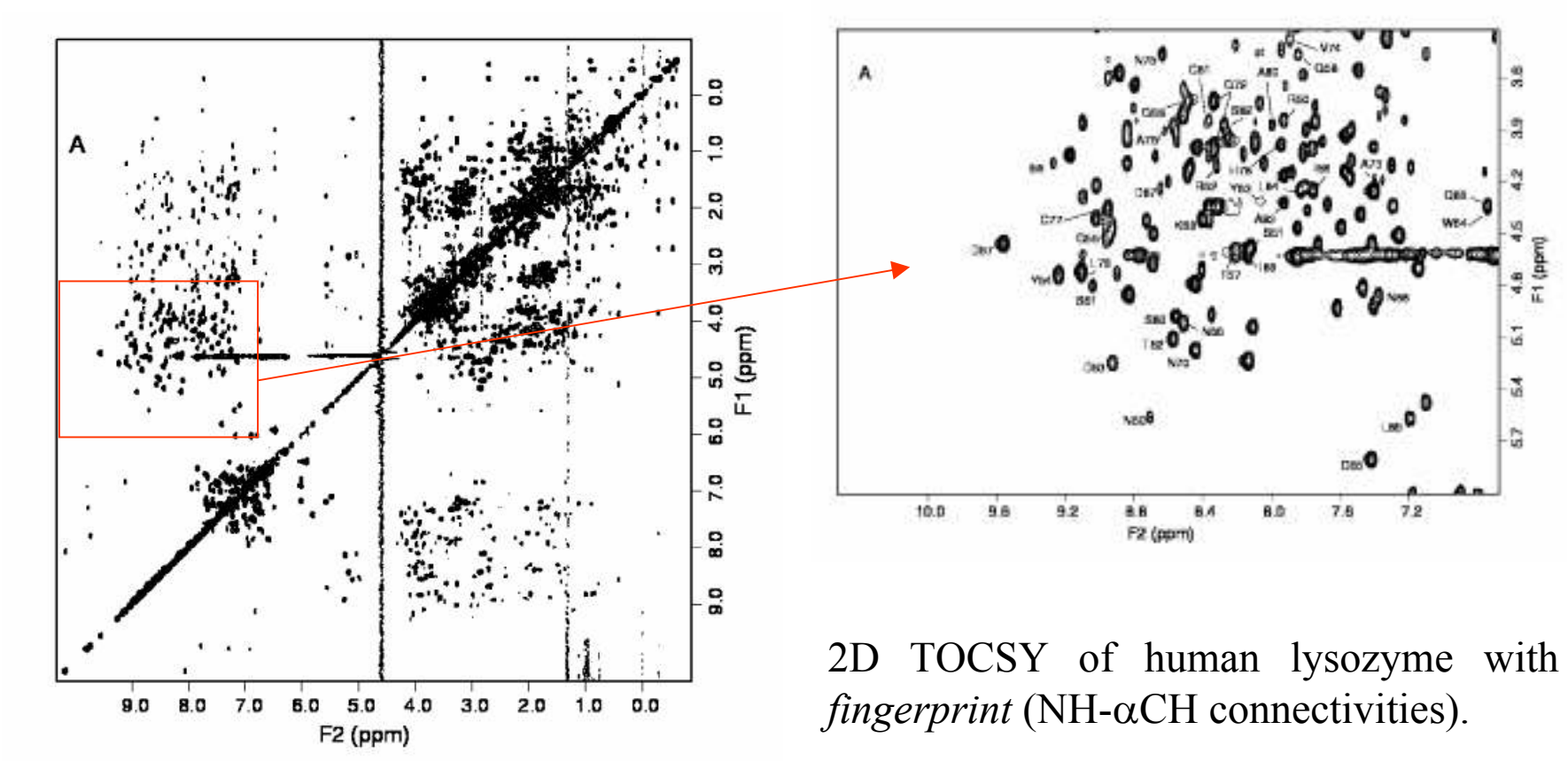
Basic features

The simplest 2D experiment is:

$$90°_x - t_1 - 90°_y - t_2$$

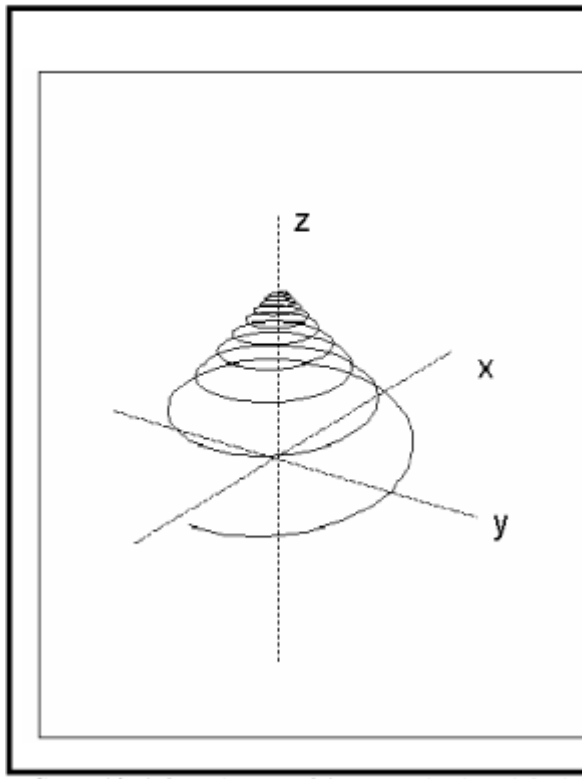


Basic features



2D TOCSY of human lysozyme with *fingerprint* (NH-αCH connectivities).

Basic features



The tranverse magnetization precesses but also goes back to the initial equilibrium position, along z, through relaxation

– relaxation + relaxation

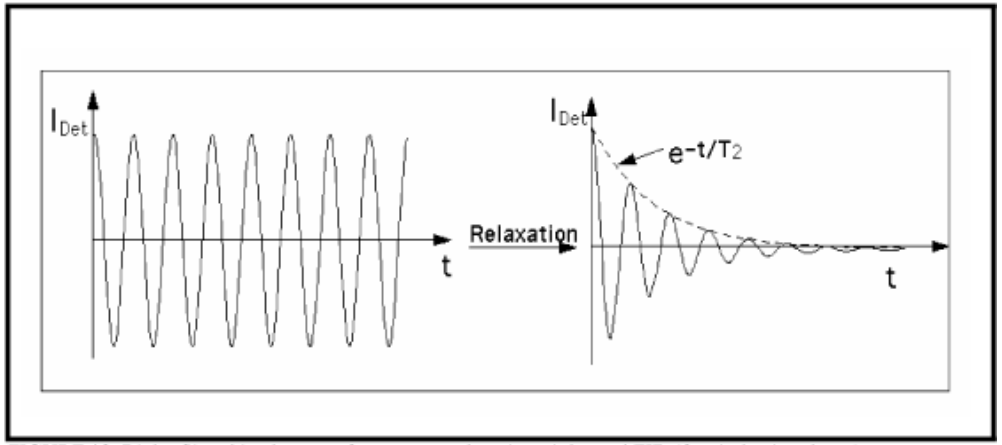
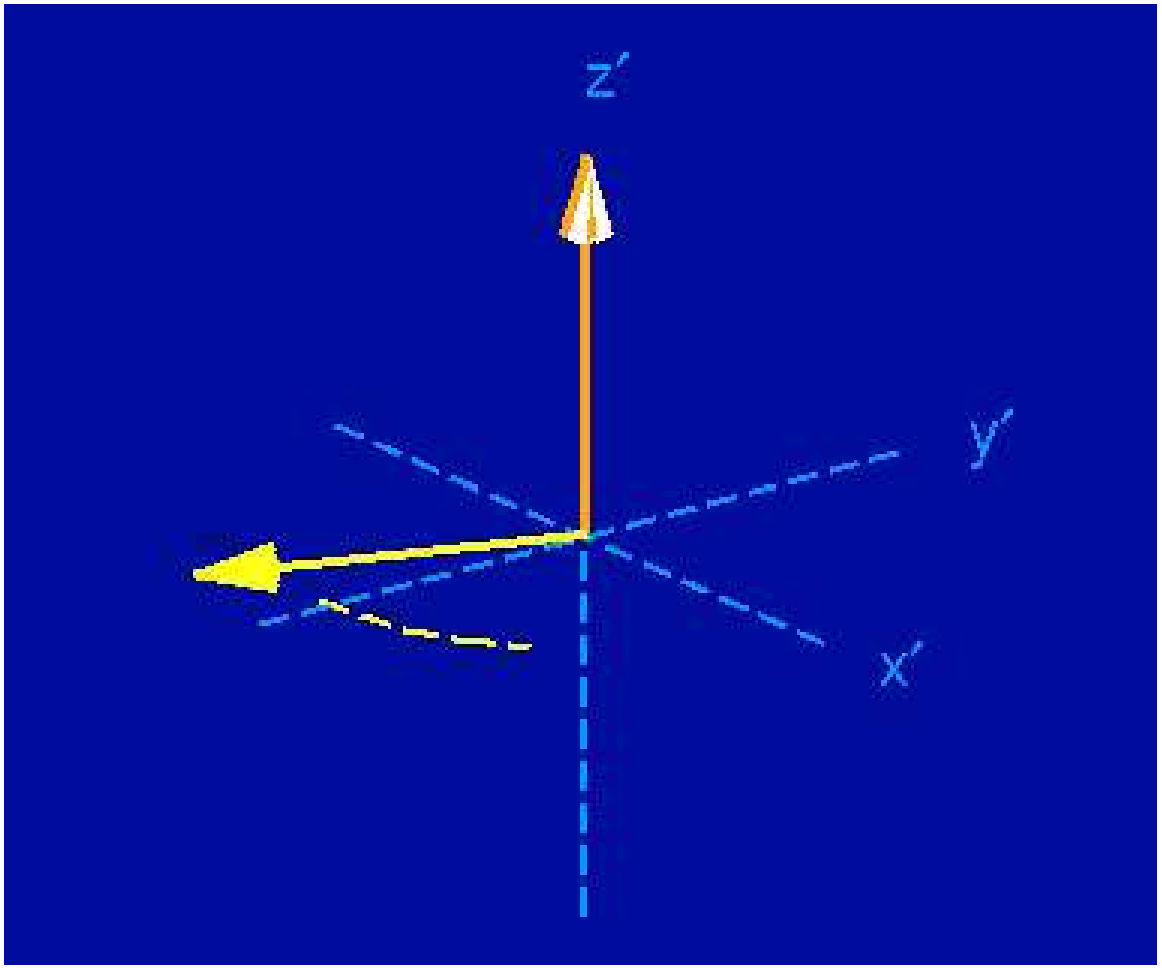


FIGURE 18. Right: Signal in absence of transverse relaxation, right: real FID (free induction decay)

FID = Free Induction Decay

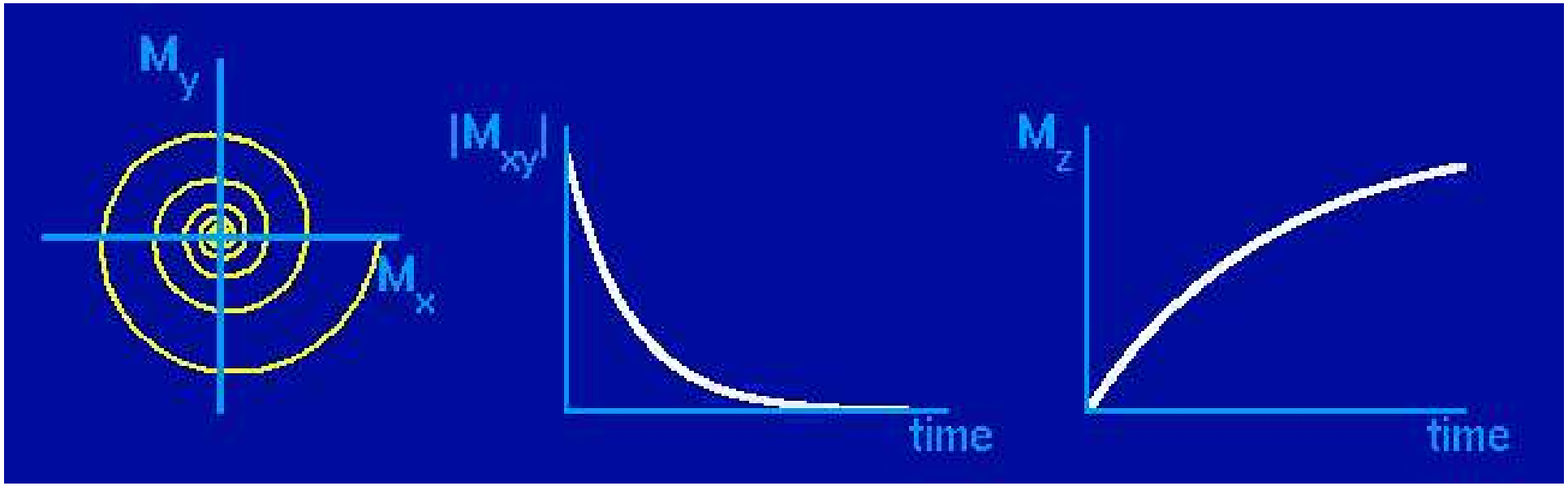
Basic features



Laboratory frame

Movie: *Brian Hargreaves*

Basic features



Relaxation = equilibrium recovery

Loss of *x*-*y* coherence = tranverse, T_2

Recovery of *z* magnetization = longitudinal, T_1

Movie: *Brian Hargreaves*

Basic features

The FID damping constant, T_2 (transverse relaxation time), can be directly interpreted as the time nesessary for a signal to lose 63% of the original intensity, and determines the signal linewidth at half-height::

$$\Delta\nu_{1/2} = \frac{1}{\pi T_2}$$

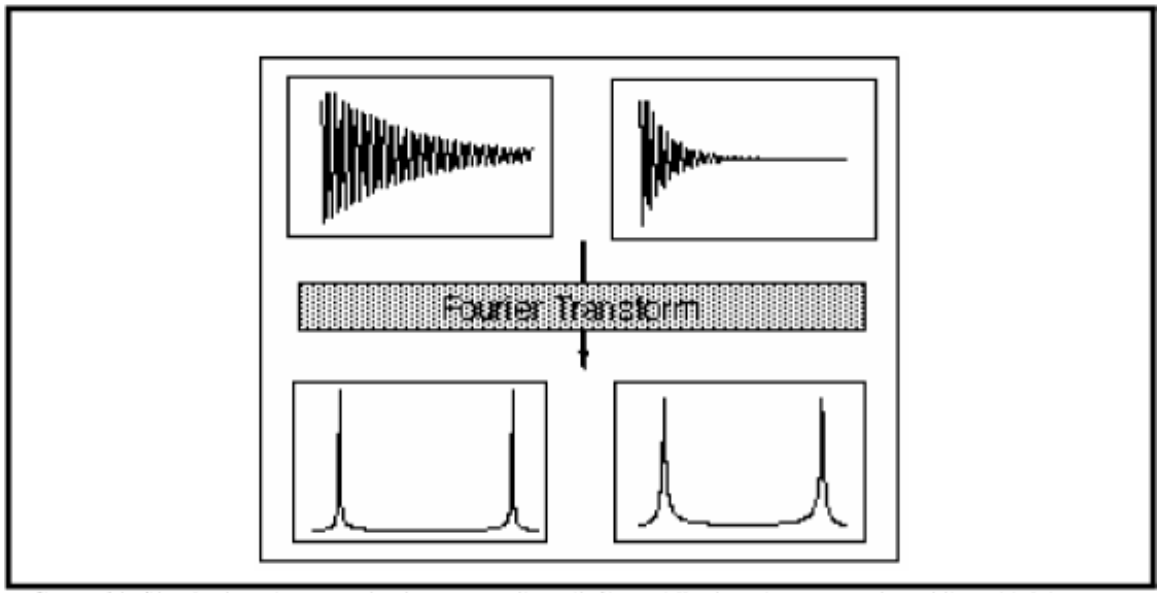
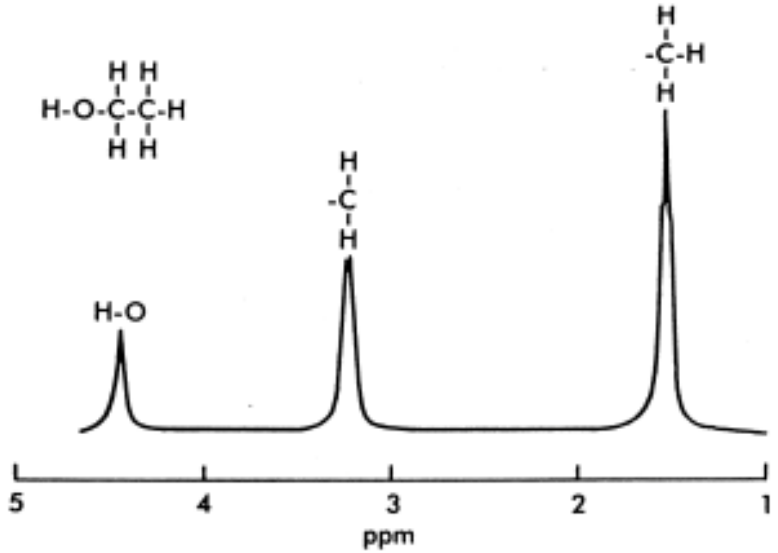


FIGURE 21. Slowly decaying FIDs lead to narrow lines (left), rapidly decaying ones to broad lines (right).

Chemical shift

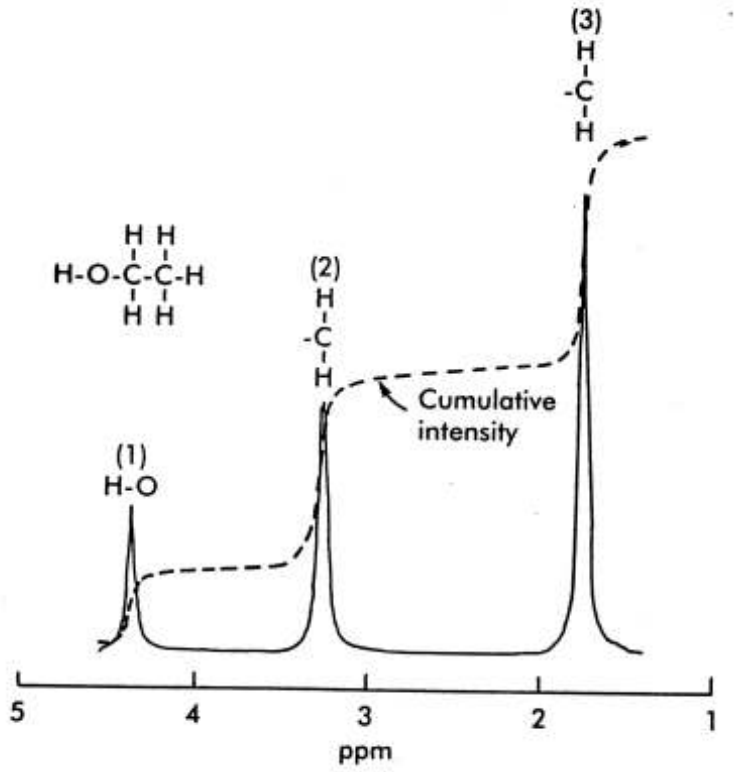
NMR signals are observed over restricted spectral windows (SW) - from hundreds of Hz up to tens of kHz - around the fundamental frequency of a nucleus.

At B = 1 T, SW = 500 Hz, the low resolution ^{1}H NMR spectrum of ethanol shows three signals, from the three different types of hydrogens

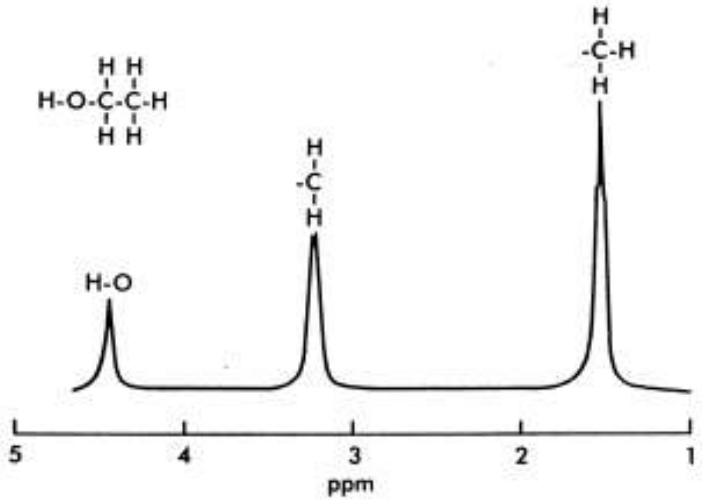


Chemical shift

Each signal exhibits an amplitude that is proportional to the concentration of the parent nuclei. Hence, for the ethanol molecule, the integral ratios of the signals will be the stoichiometric coeffiecients of the basic formula.



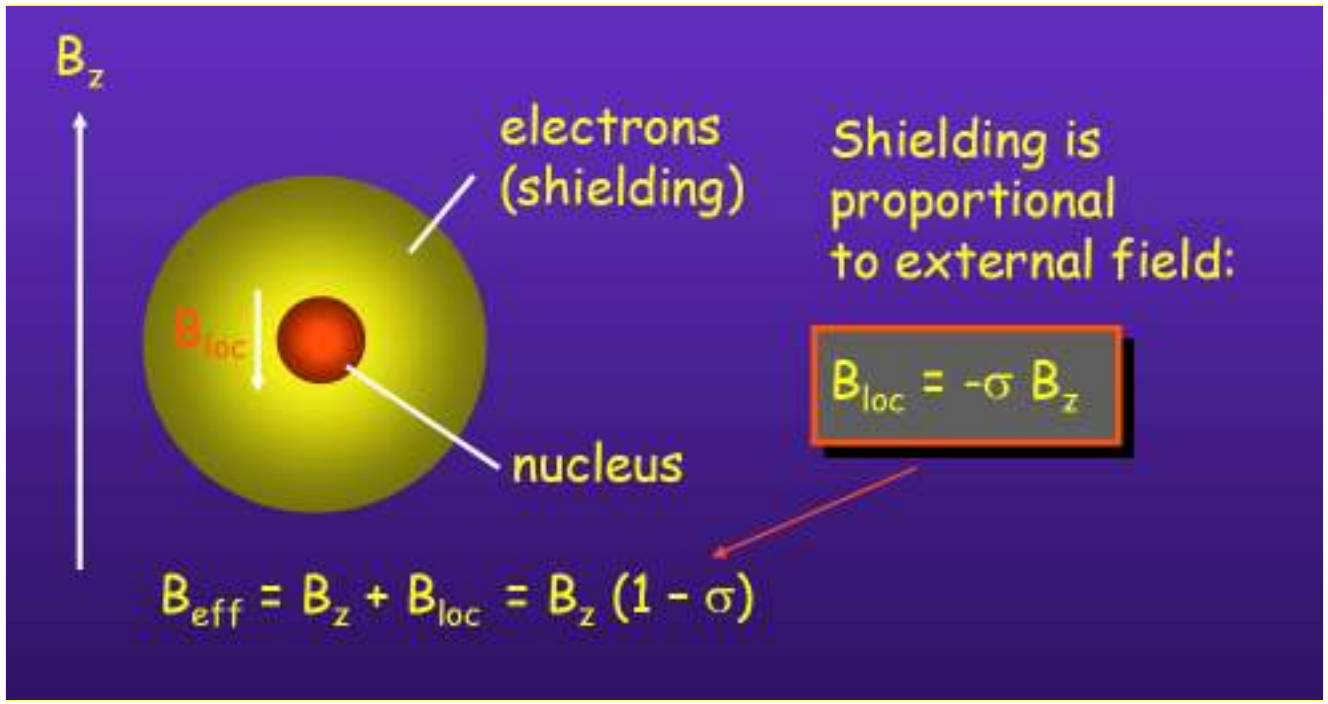
Chemical shift



The ethanol hydrogen nuclei resonate at *different* frequencies in *different* chemical environment - their resonance frequencies show a *chemical shift*.

In general, chemical shifts are observed in any molecule and for any type of nucleus.

Chemical shift



The chemical shift depends on the actual external magnetic field shielding that a nucleus experiences due to the local enviroment.

The fundamental resonance condition is recast to include the screening term that changes the resonance frequency or chemical shift

$$\nu_i = \left|\frac{\gamma}{2\pi}\right| B_0 \left(1 - \sigma_i\right)$$

where σ_i is the shield constant of nucleus *i*.

The shield constant collects the effect of several contributions such as the diamagnetic (dia), the paramagnetic (para), the chemical shift anisotropy (csa), the ring currents (rc), the electric field (ef), the solvent (solv):

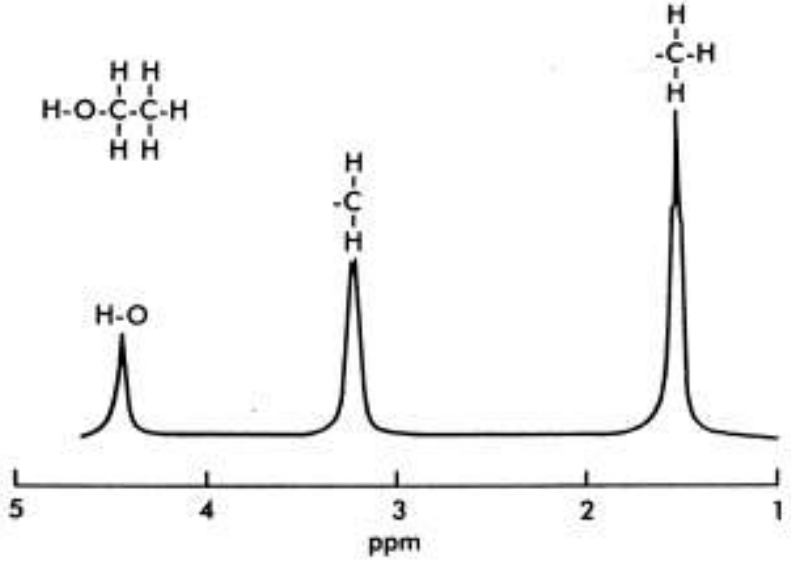
$$\sigma = \sigma_{dia} + \sigma_{para} + \sigma_{csa} + \sigma_{rc} + \sigma_{ef} + \sigma_{solv}$$

Chemical shift

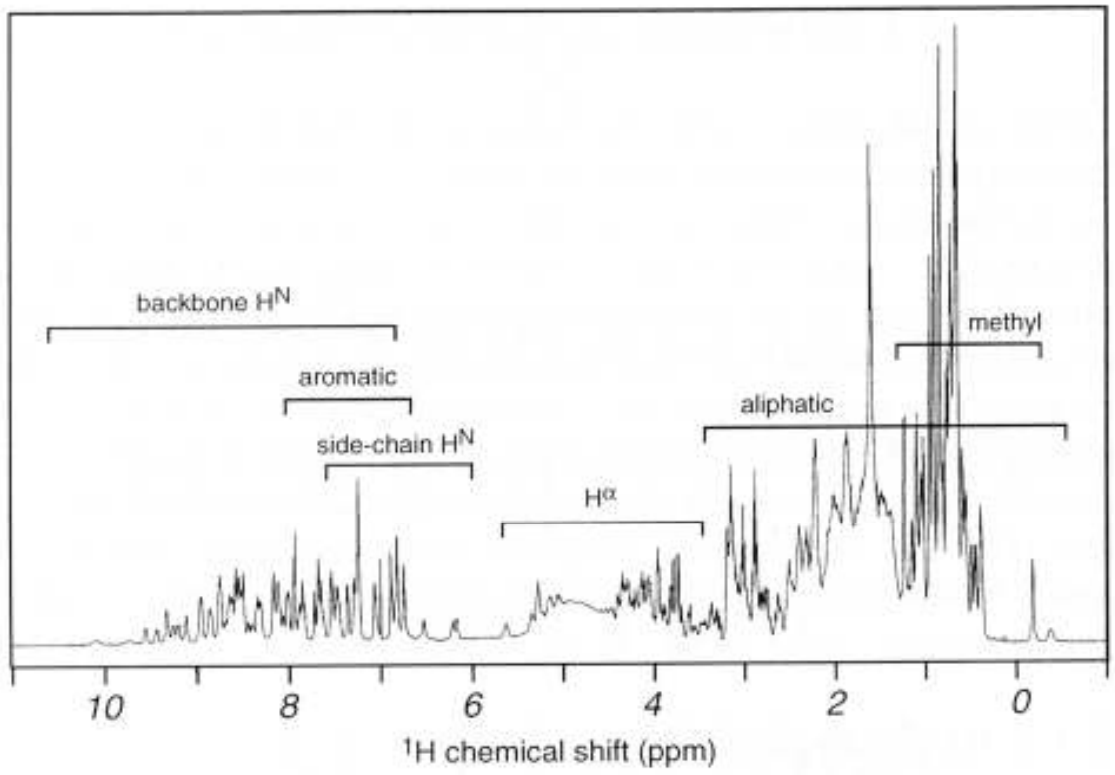
Chemical shifts (δ) are measured in parts per million, ppm, with respect to a reference compound frequency that sets the scale origin ($\delta = 0$ ppm), according to:

f = absolute signal frequency
f_0 = absolute reference frequency

$$\delta = \frac{f - f_0}{f_0} \times 10^6 \text{ ppm}$$

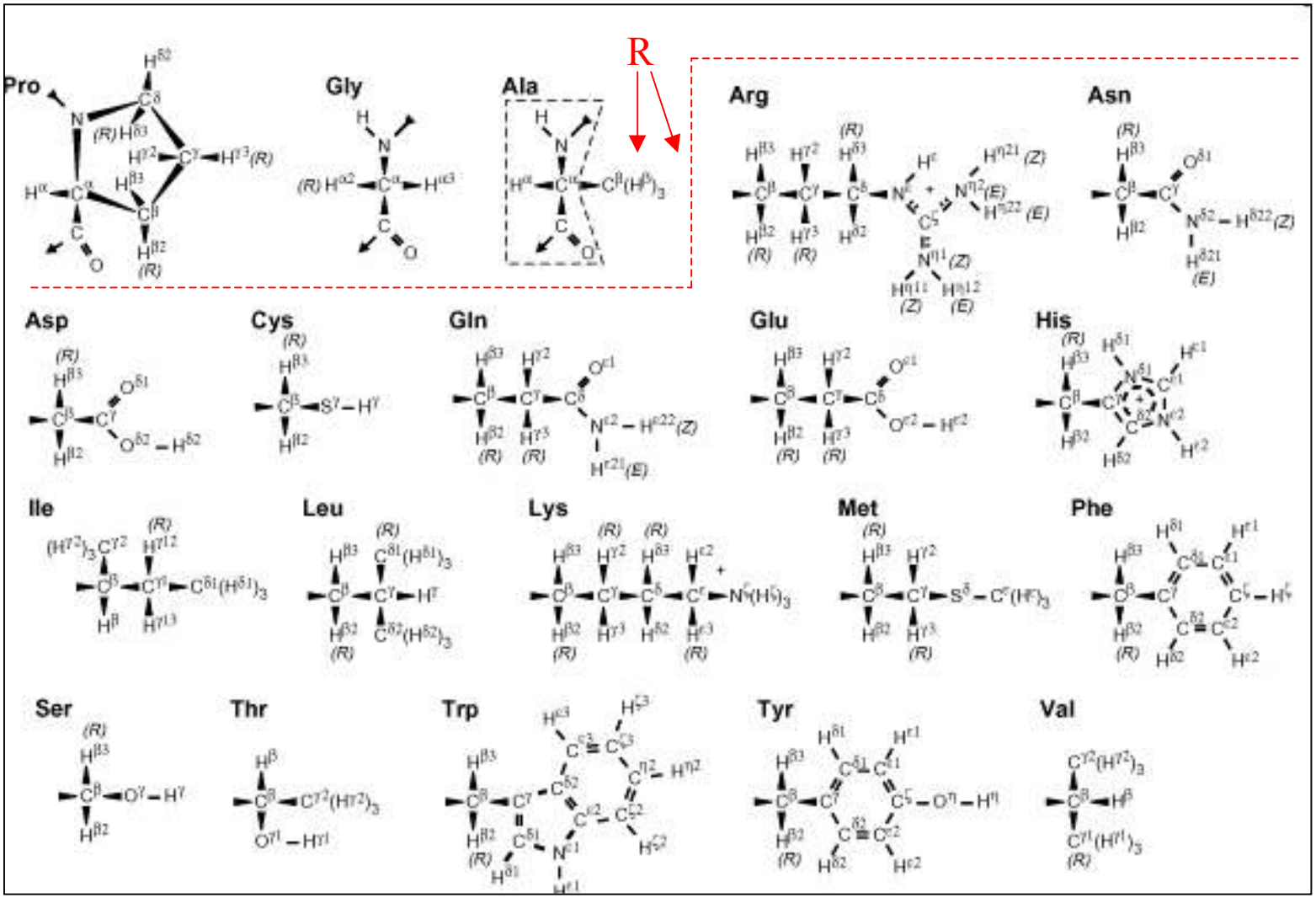


Chemical shifts are very important to interpret the NMR spectra of biopolymers such as proteins and oligonucleotides.



^{1}H NMR spectrum of a folded protein

Chemical shift



For any aminoacid (R = side chain), it is possible to establish limiting δ values.

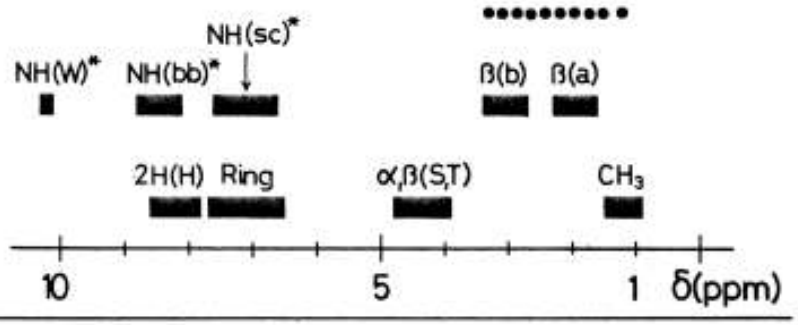
Chemical shift

TABLE 2.3. Random Coil ^{1}H Chemical Shifts for the 20 Common Amino Acid Residues[a]

Residue	NH	αH	βH	Others
Gly	8.39	3.97		
Ala	8.25	4.35	1.39	
Val	8.44	4.18	2.13	γCH_3 0.97, 0.94
Ile	8.19	4.23	1.90	γCH_2 1.48, 1.19
				γCH_3 0.95
				δCH_3 0.89
Leu	8.42	4.38	1.65,1.65	γH 1.64
				δCH_3 0.94, 0.90
Pro[b]		4.44	2.28,2.02	γCH_2 2.03, 2.03
				δCH_2 3.68, 3.65
Ser	8.38	4.50	3.88,3.88	
Thr	8.24	4.35	4.22	γCH_3 1.23
Asp	8.41	4.76	2.84,2.75	
Glu	8.37	4.29	2.09,1.97	γCH_2 2.31, 2.28
Lys	8.41	4.36	1.85,1.76	γCH_2 1.45, 1.45
				δCH_2 1.70, 1.70
				ϵCH_2 3.02, 3.02
				ζNH_3 7.52
Arg	8.27	4.38	1.89,1.79	γCH_2 1.70, 1.70
				δCH_2 3.32, 3.32
				NH 7.17, 6.62
Asn	8.75	4.75	2.83,2.75	γNH_2 7.59, 6.91
Gln	8.41	4.37	2.13,2.01	γCH_2 2.38, 2.38
				δNH_2 6.87, 7.59
Met	8.42	4.52	2.15,2.01	γCH_2 2.64, 2.64
				ϵCH_3 2.13
Cys	8.31	4.69	3.28,2.96	
Trp	8.09	4.70	3.32,3.19	2H 7.24
				4H 7.65
				5H 7.17
				6H 7.24
				7H 7.50
				NH 10.22
Phe	8.23	4.66	3.22,2.99	2,6H 7.30
				3,5H 7.39
				4H 7.34
Tyr	8.18	4.60	3.13,2.92	2,6H 7.15
				3,5H 6.86
His	8.41	4.63	3.26,3.20	2H 8.12
				4H 7.14

[a] Data for the nonterminal residues X in tetrapeptides GGXA, pH 7.0, 15°C (from Bundi and Wüthrich (1979a), except that more precise data were obtained for Leu, Pro, Lys, Arg, Met, and Phe using new measurements at 500 MHz).
[b] Data for trans-Pro.

TABLE 2.4. Groups of Hydrogen Atoms in the Common Amino Acid Residues with Similar Random Coil ^{1}H Chemical Shifts[a]

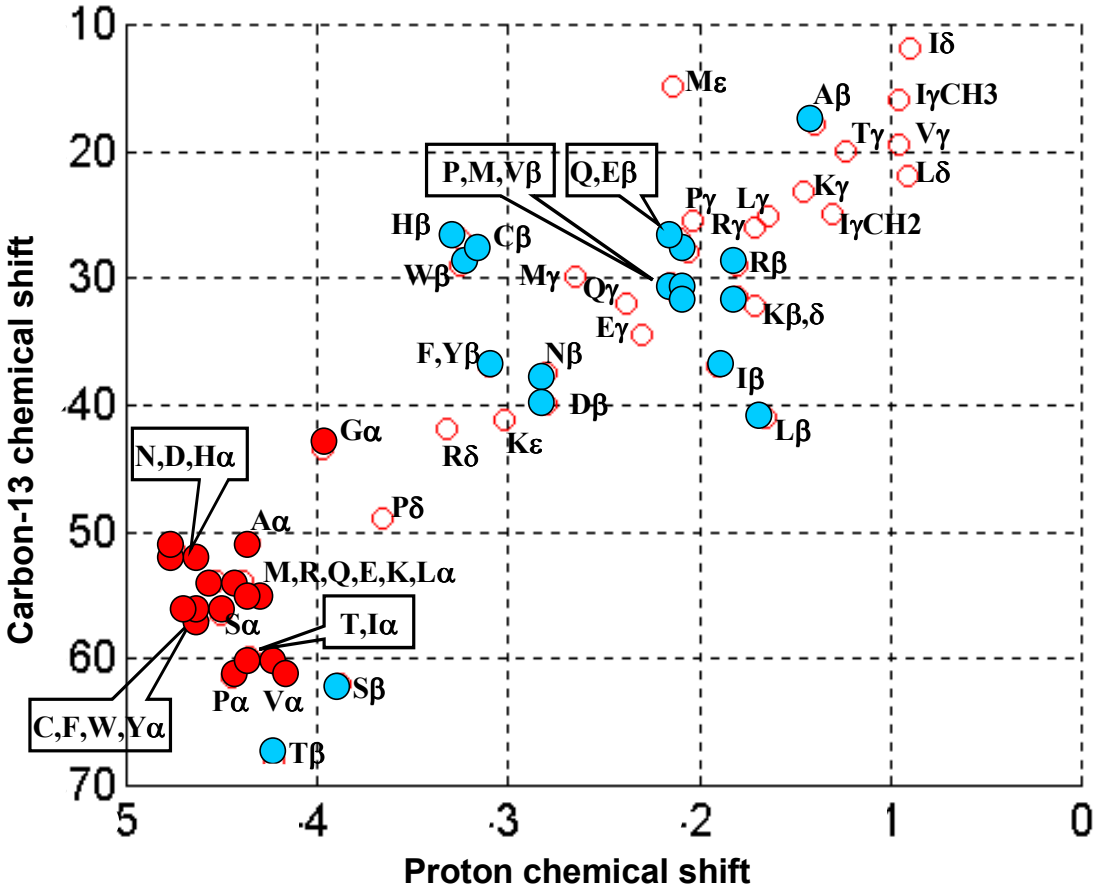


Code	δ (ppm)	Comments
CH_3	0.9–1.4	
β(a)	1.6–2.3	βH of V,I,L,E,Q,M,P,R,K
β(b)	2.7–3.3	βH of C,D,N,F,Y,H,W
••••	1.2–3.3	Other Aliphatic CH
α,β(S,T)	3.9–4.8	All αH, βH of S and T
Ring	6.5–7.7	Aromatic CH of F,Y,W; 4H of H
2H(H)	7.7–8.6	2H of H in the pH range 1–11
NH(sc)*	6.6–7.6	Side Chain NH of N,Q,K,R
NH(bb)*	8.1–8.8	Backbone NH
NH(W)*	10.2	Indole NH of W

[a] In model peptides the labile protons (identified by *) are only observed in H_2O solution. The singlet resonance of ϵCH_3 in Met is at 2.13 ppm (Table 2.3).

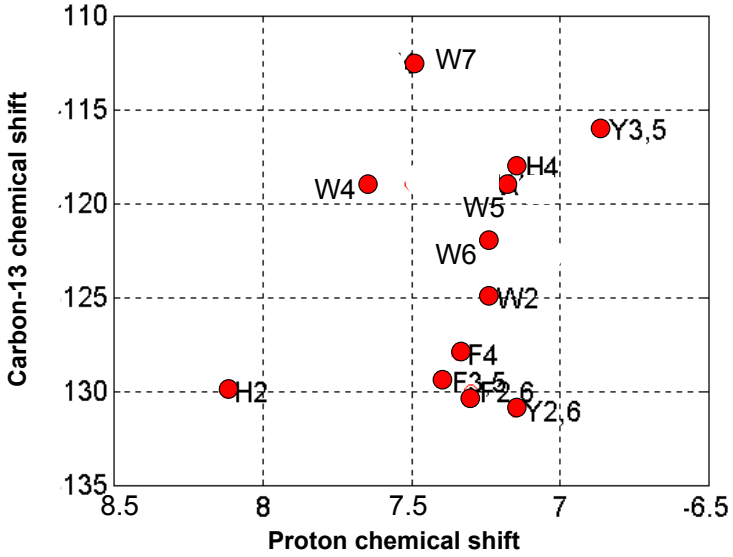
The limiting δ values are expected for disordered structures, *random coil*.

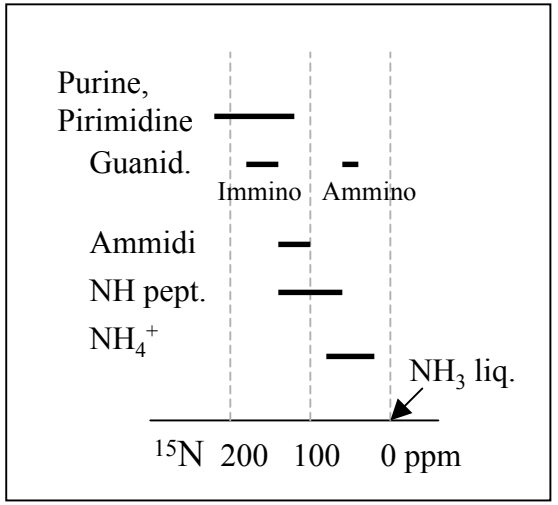
Chemical shift



The limiting δ values for disordered structures can be determined also for nuclei other than ^{1}H.

Chemical shift

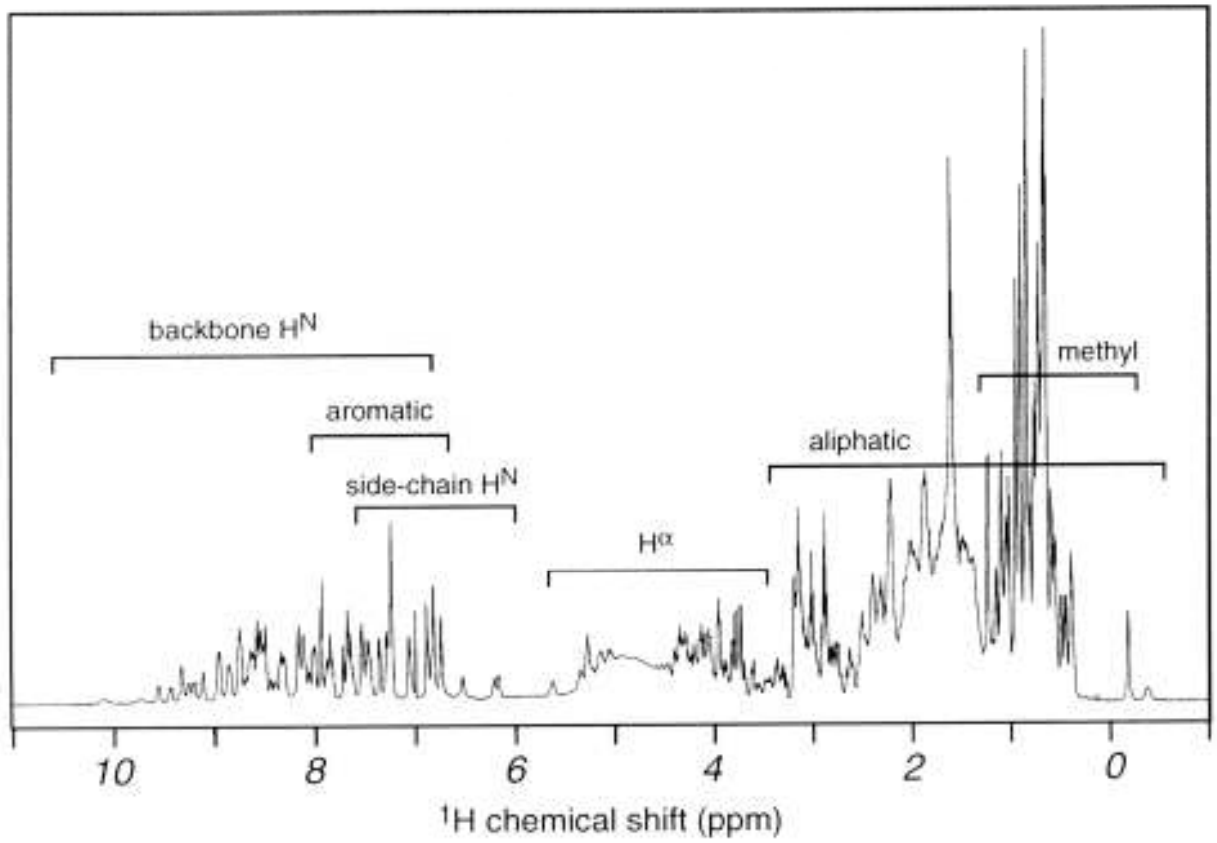




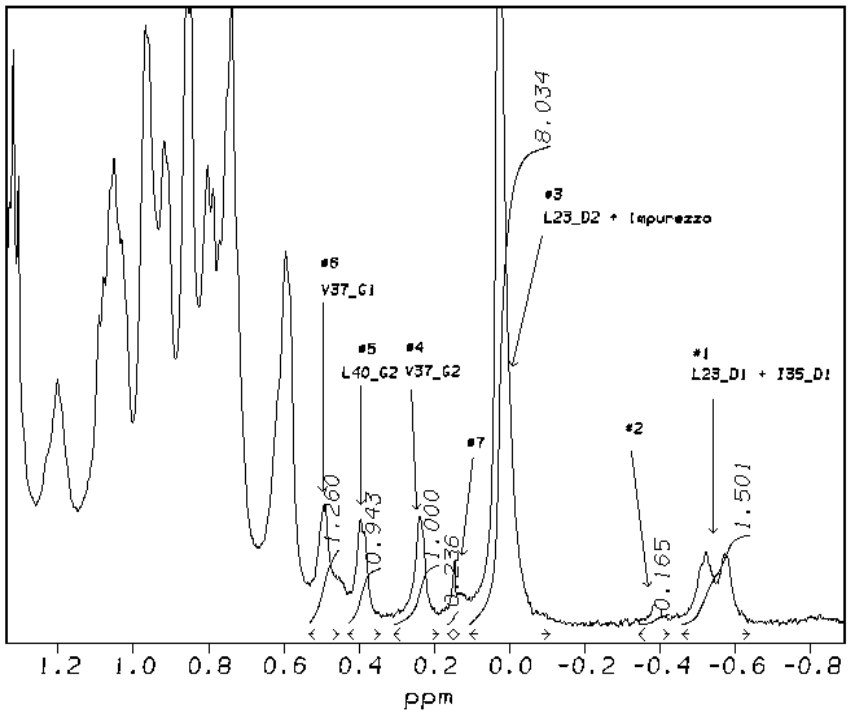
The limiting δ values for disordered structures can be determined also for nuclei other than ^{1}H.

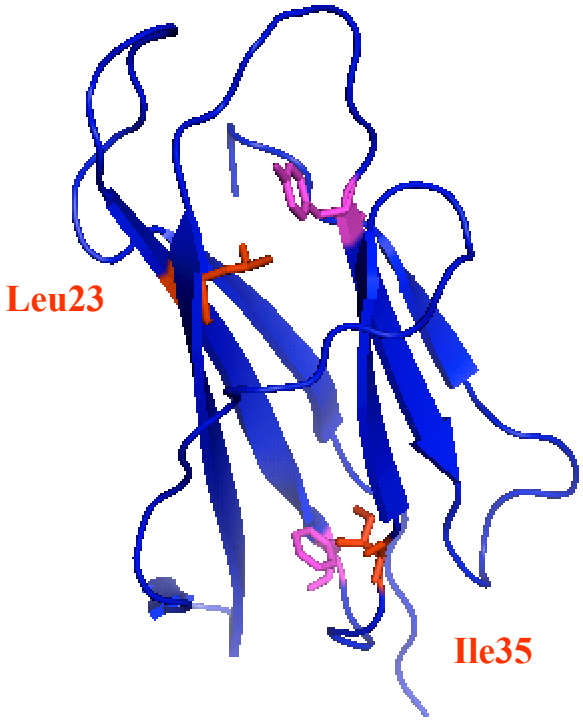
The secondary and tertiary structure in a polypeptide chain change sensibly the local chemical environment, and the chemical shifts deviate from the limiting values of disordered structures.

Chemical shift



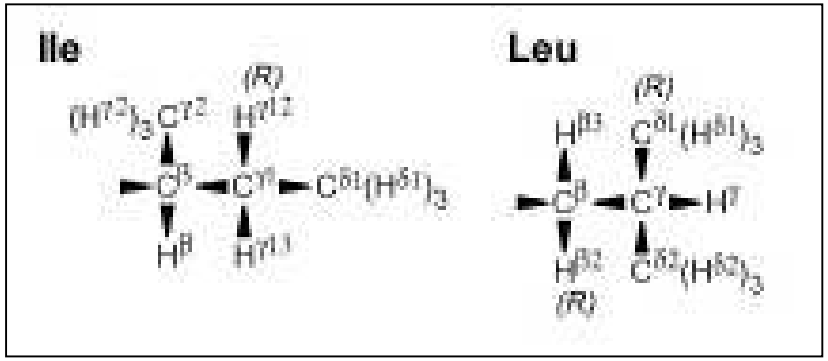
Chemical shift





Effect of tertiary structure on the chemical shifts

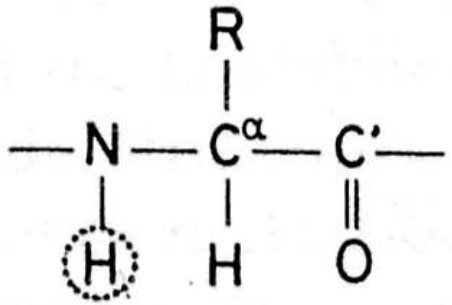
Chemical shift



	NH	α	β	γ		δ
Ile	8.19	4.23	1.90	1.48, 1.19	γCH_3 0.95	0.89
Leu	8.42	4.38	1.65, 1.65	1.64		0.94, 0.90

Also the secondary structure of a polypeptide chain introduces deviations of the chemical shifts from the limiting values of disordered structures.

Contrary to the deviations due to tertiary structure, the chemical shift deviations from secondary structure are quite diagnostic and affect essentially only the peptide backbone nuclei.



Chemical shift

The deviation from the limiting values of the αH chemical shift is employed to define the *chemical shift index*, CSI, of a protein. The deviation is meaningful when > ±0.1 ppm with respect to the random coil value.

TABLE VIII
AVERAGE SECONDARY SHIFT FOR VARIOUS NUCLEI RELATIVE TO RANDOM COIL VALUES[a]

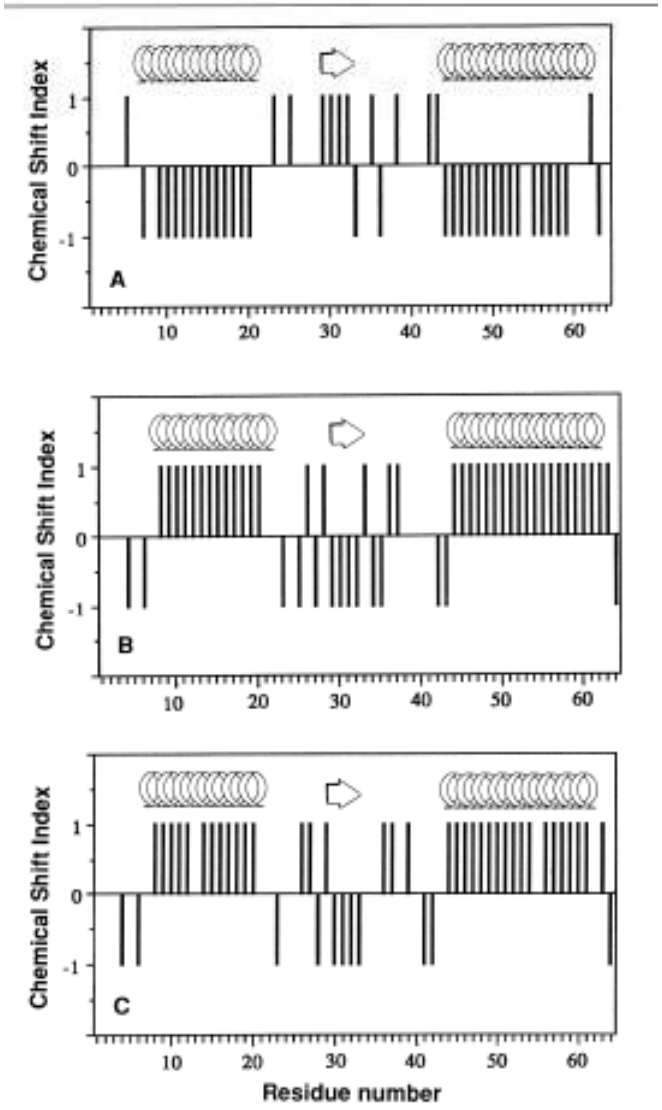
Nucleus	Helix	β Strand
α-^{1}H	−0.38	0.38
N-^{1}H	−0.19	0.29
2-^{13}C	2.6	−1.4
1-^{13}C	1.7	−1.4
^{15}N	−1.7	1.2

[a] Data are given in ppm.

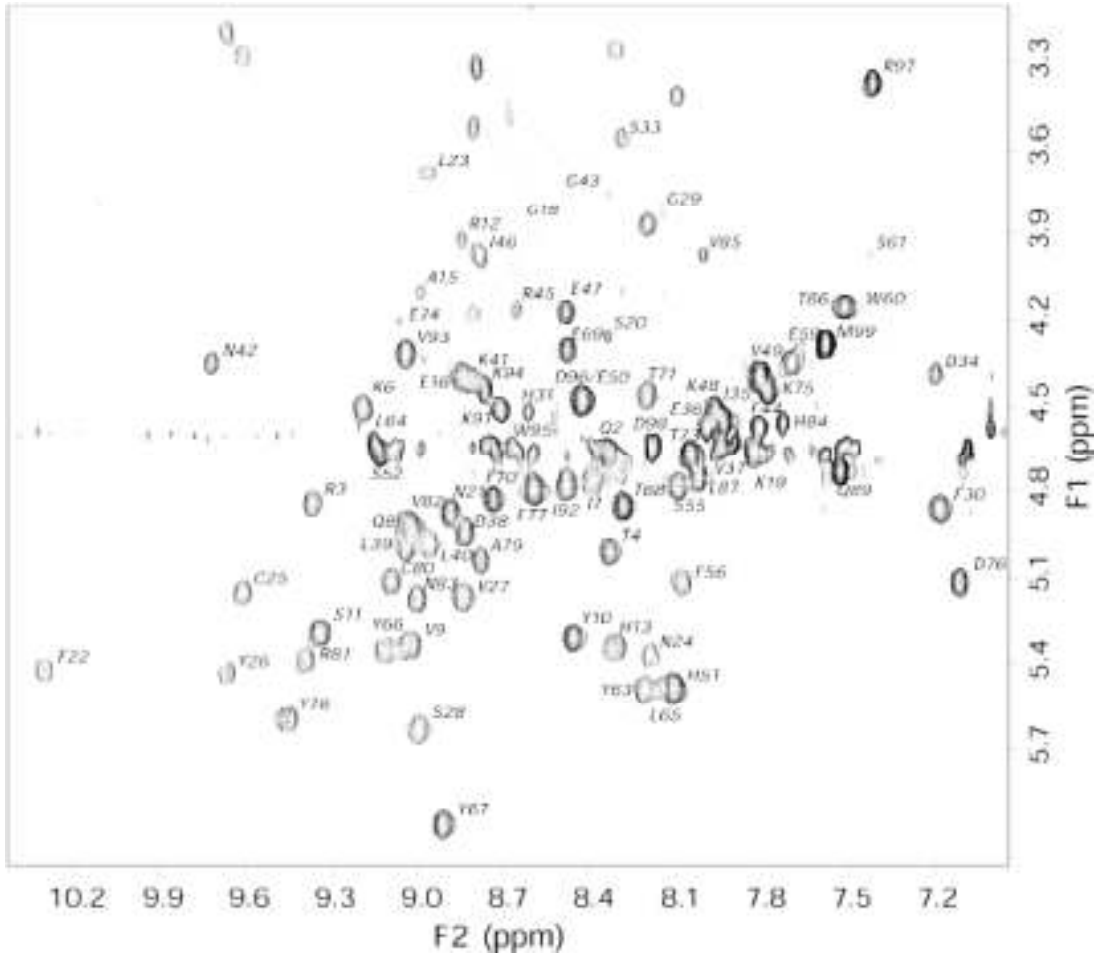
H^{α}, H^{N}

C^{α}

C’



Chemical shift



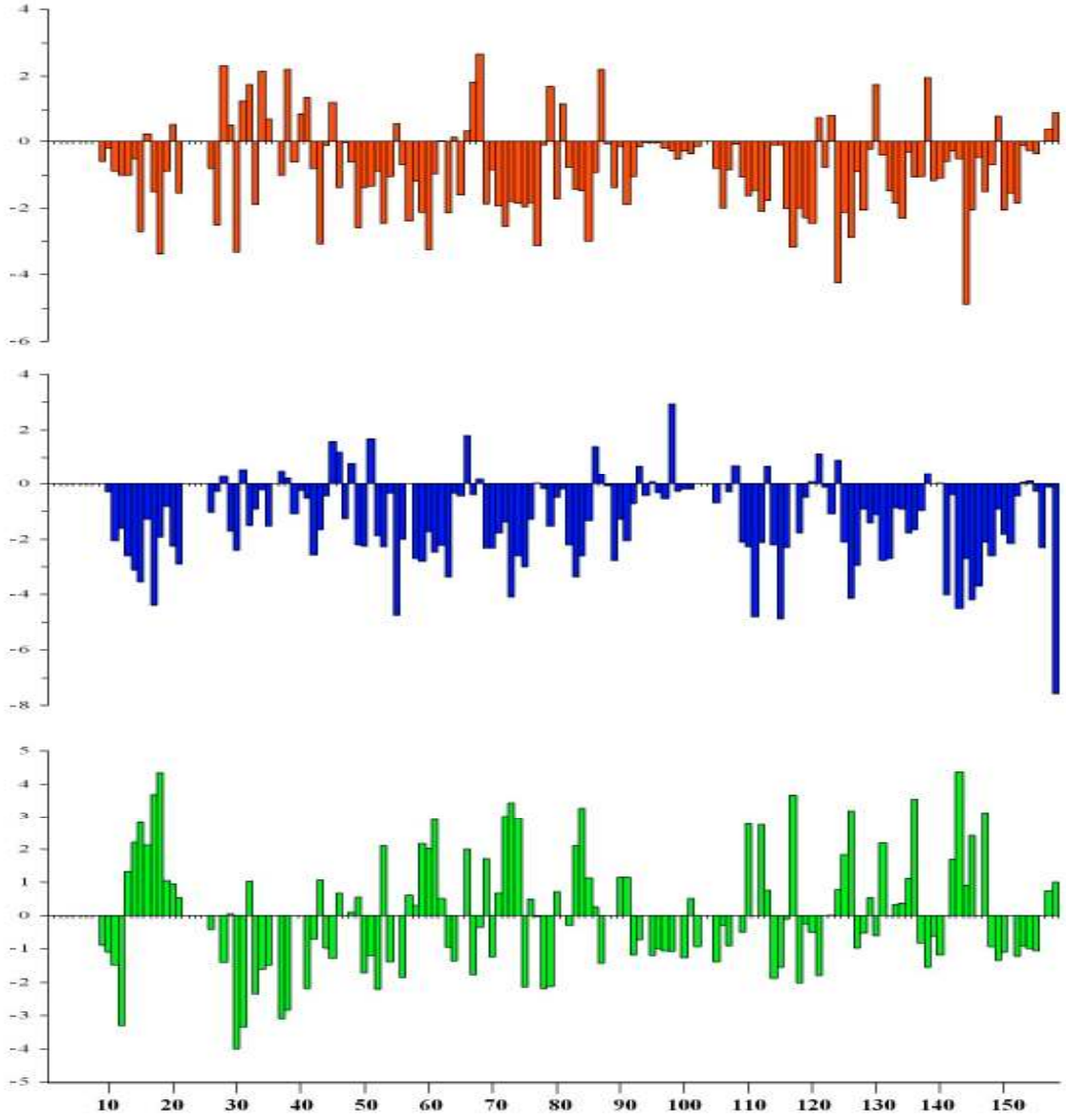
Fingerprint of β2-m 2D ^{1}H TOCSY

Chemical shift

$\Delta\delta\ ^{13}C^{\alpha}$ (ppm)

$\Delta\delta\ ^{13}C'$ (ppm)

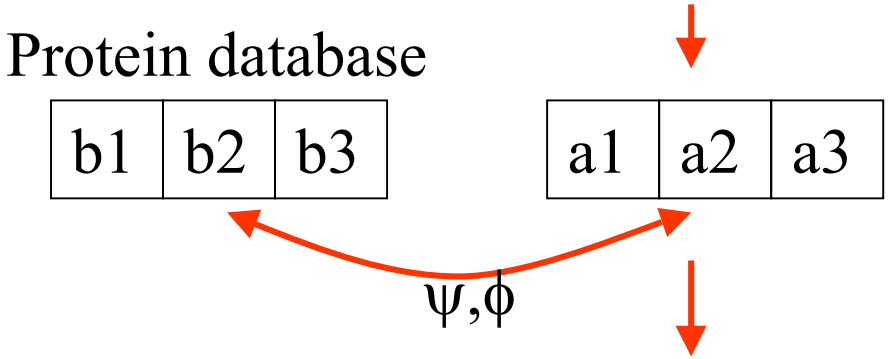
$\Delta\delta\ ^{13}C^{\beta}$ (ppm)



TALOS predictions of ψ and ϕ bb torsion angle

•aa sequence

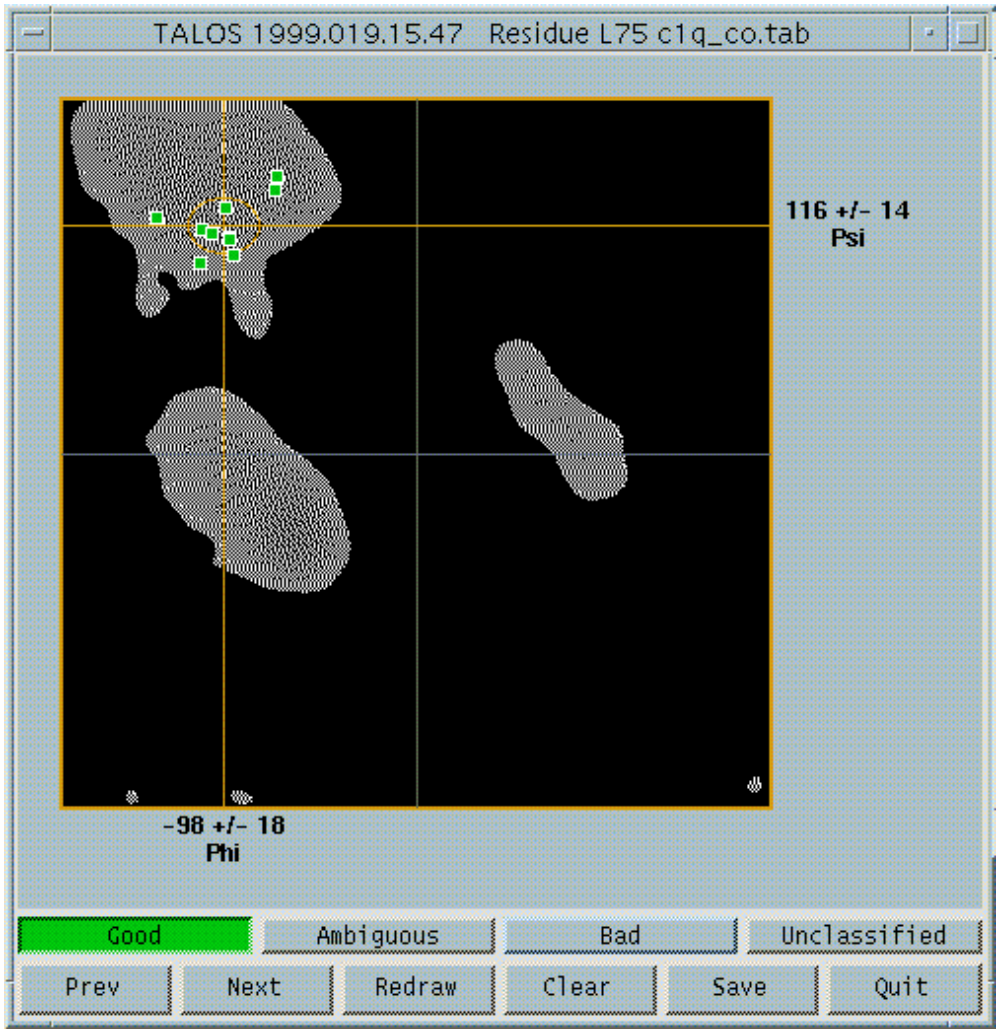
•chemical shifts (H^{α}, C^{α}, C^{β}, C', $^{15}N^{H}$)

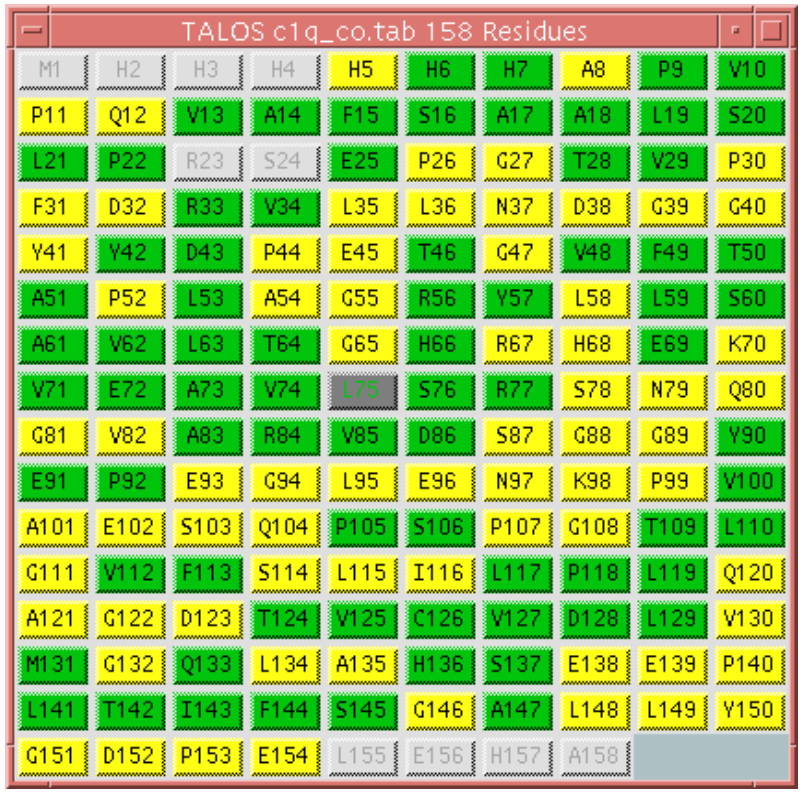


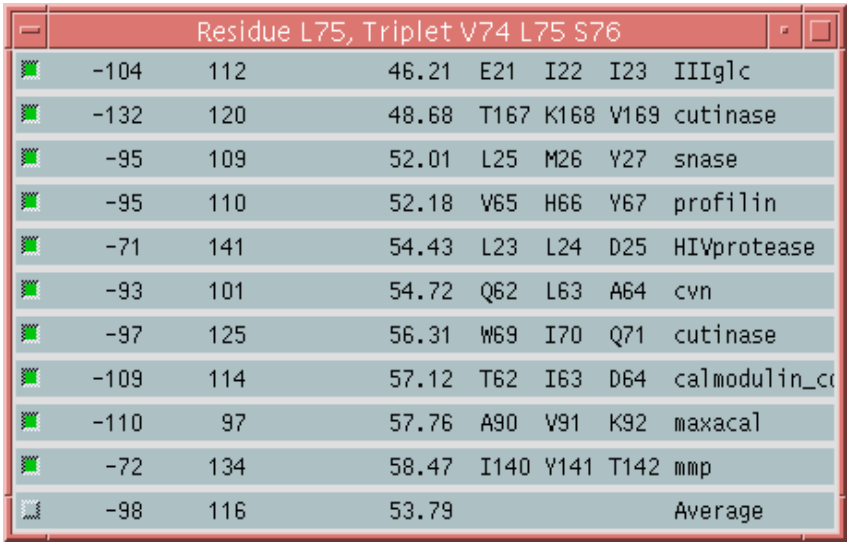
Average of ψ,ϕ values and st.dev. of the ten triplets with highest degree of similarity

Inspection of predictions and selection of the useful ones

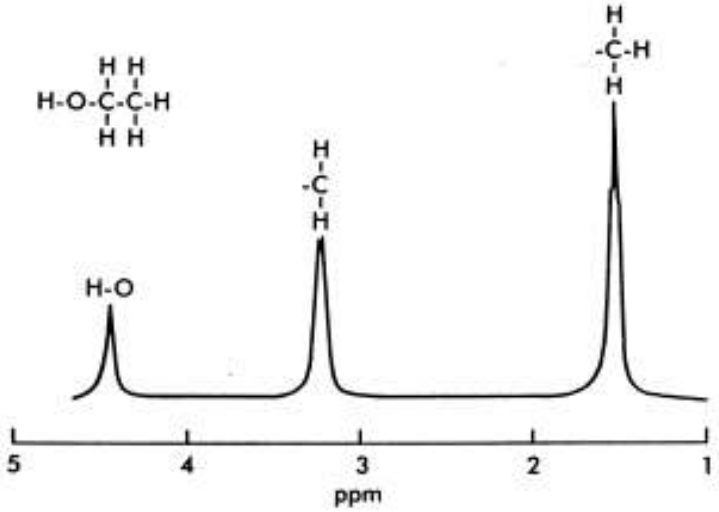
Chemical shift



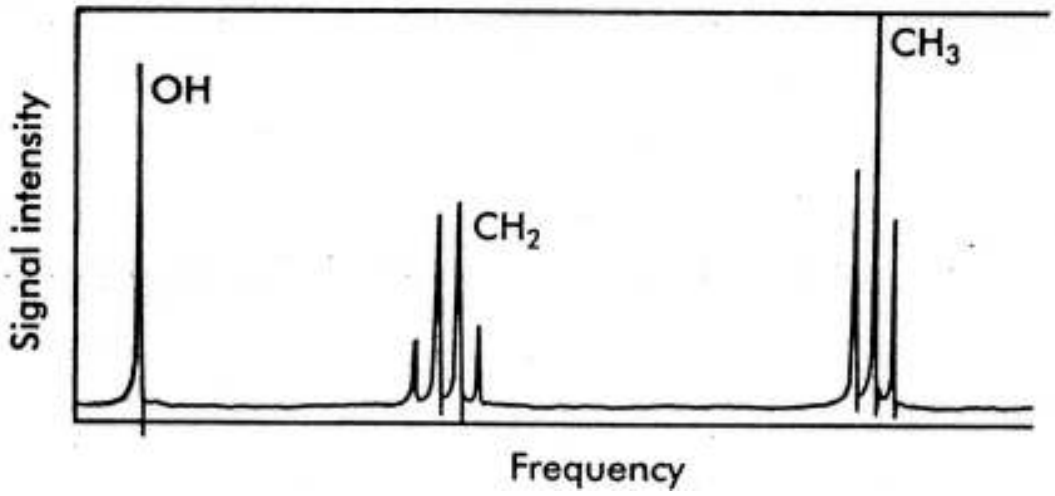




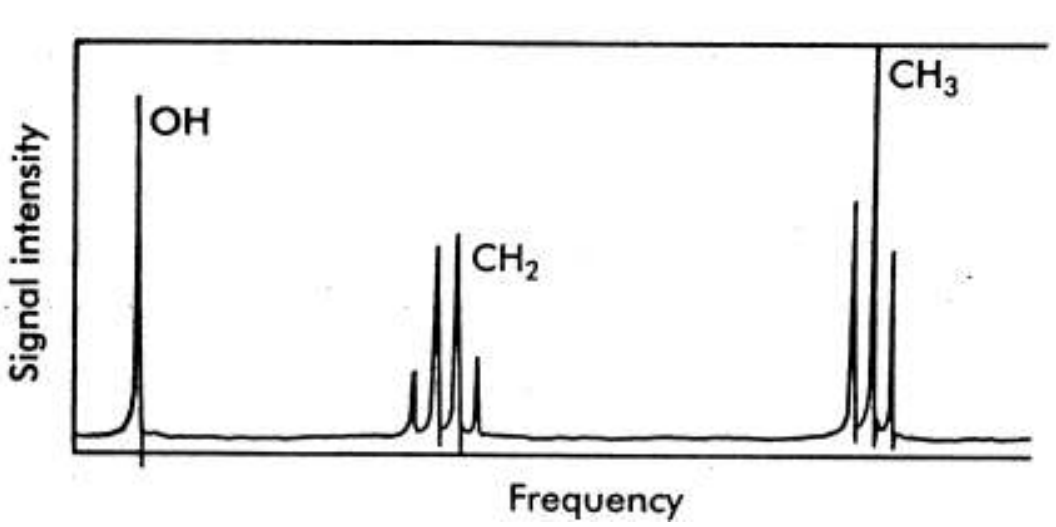
Scalar coupling



The ^{1}H NMR spectrum of ethanol reveals an interesting *fine structure* when run at high resolution.



Scalar coupling



Why the methyl signal has a triplet structure?

Why the methylene is a quartet?

Why the hydroxyl is a singlet?

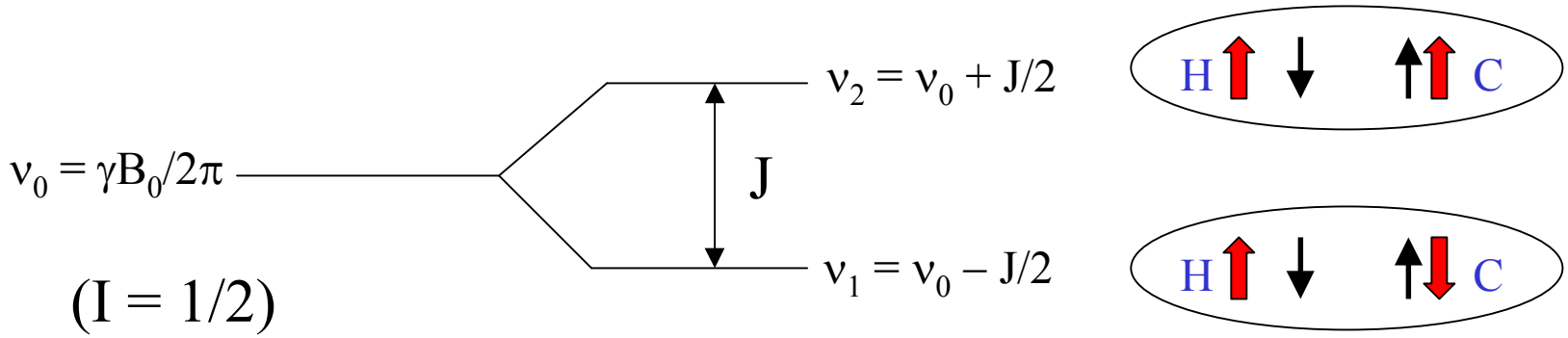
Scalar coupling

The magnetic dipole of a nuclear spin (↑) is able to *feel* the spin state of another nucleus belonging to the same molecule. The information is passed through the spin state of the electrons that form the chemical bonds (↓).

Following Hund's rule, the electrons with orbital density at the nucleus other than zero (s type) have an antiparallel spin with respect to the nuclear spin, in the lower energy configuration. The same electrons, involved in a σ chemical bond, obey the Pauli principle and therefore arrange with antiparallel spin in the bonding orbital. Therefore the nuclear spin state of the second nucleus determines the larger or smaller energy of the system. The transition energy of a nuclear spin (H), i.e. its transition frequency, will be therefore affected by the spin state of the bound nucleus (C).

Scalar coupling

This coupling mechanism leads to the *splitting* of the NMR transition frequency, by an extent equal to the *scalar coupling constant* or *spin-spin coupling constant*, *J*.



The value of any scalar coupling constant is independent of the external magnetic field B. Therefore *J* is expressed in Hz and not in ppm.

The scalar coupling information travels along the chemical bonds, but attenuates substantially beyond three chemical bonds.

Scalar coupling

Multiplicity

Each single scalar coupling with a spin 1/2 nucleus introduces a doubling of the number of lines. This comes from the basic multiplicity rule = $(2 \times I + 1) = (2 \times 1/2 + 1)$.

However it is necessary to distinguish between coupling with equivalent nuclei and non-equivalent nuclei.

If N nuclei with I = 1/2 are coupled to nucleus x, the multiplicity of the x signal will be:

2^N if the N nuclei are ***not equivalent***;

(N+1) if the N nuclei are ***equivalent***

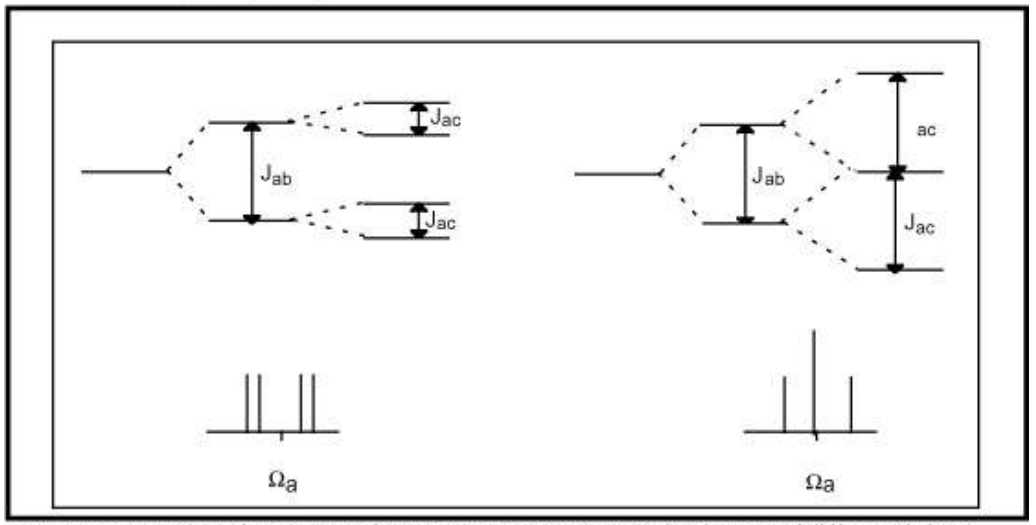
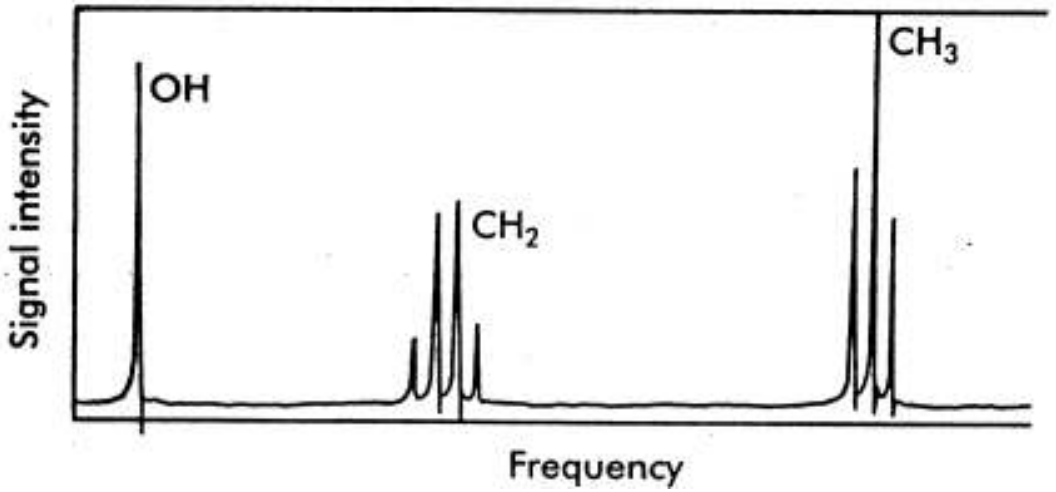


FIGURE 17. Coupling of a proton a with two other protons b and c for the case of different (left) or similar (right) couplings.

Scalar coupling



We should understand why the CH_3 signal is a triplet and the CH_2 is a quartet.

Why the hydroxyl is a singlet?

Scalar coupling

The coupling constant of vicinal nuclei (^3J) show a characteristic dependence on the dihedral angle between the involved nuclei.

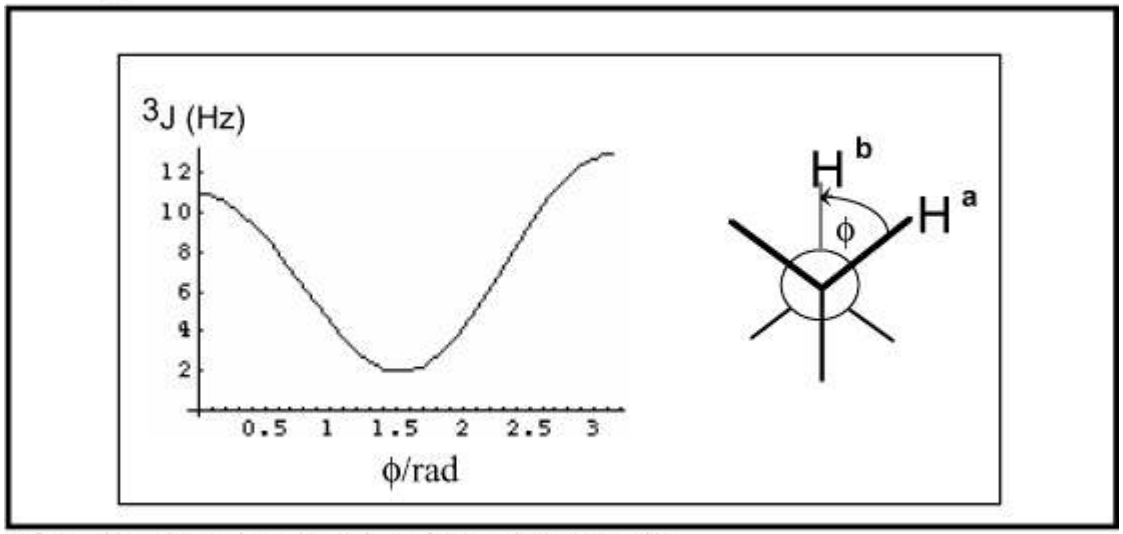


FIGURE 12. Left: Karplus curve, right: Definition of dihedral angles

$^3J = 7 - 1.0\cos\phi + 5\cos 2\phi$ for ^{1}H-^{1}H couplings

$^3J = 3.81 - 0.9\cos\phi + 3.83\cos 2\phi$ for ^{1}H-^{13}C couplings

Karplus equations

Scalar coupling

The dependence of ^{3}J on the dihedral angle is due to the extent of overlap of the binding orbitals that propagate the spin-spin coupling information.

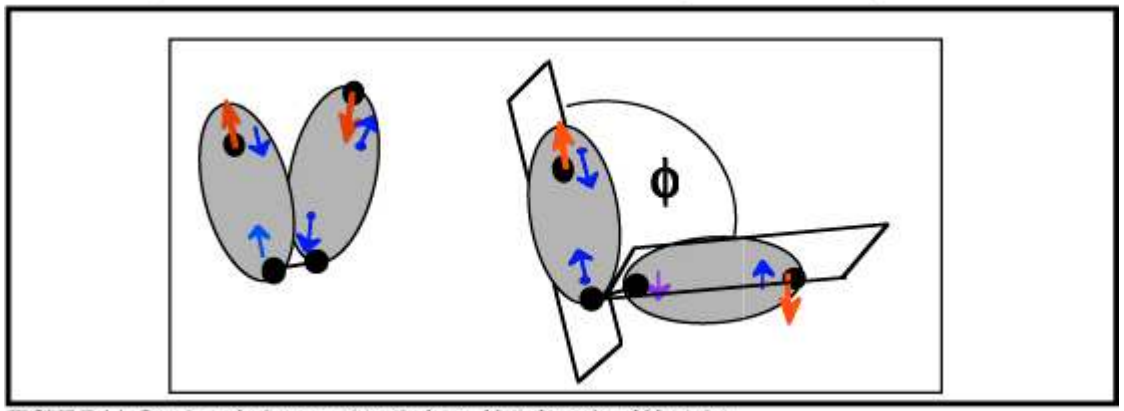
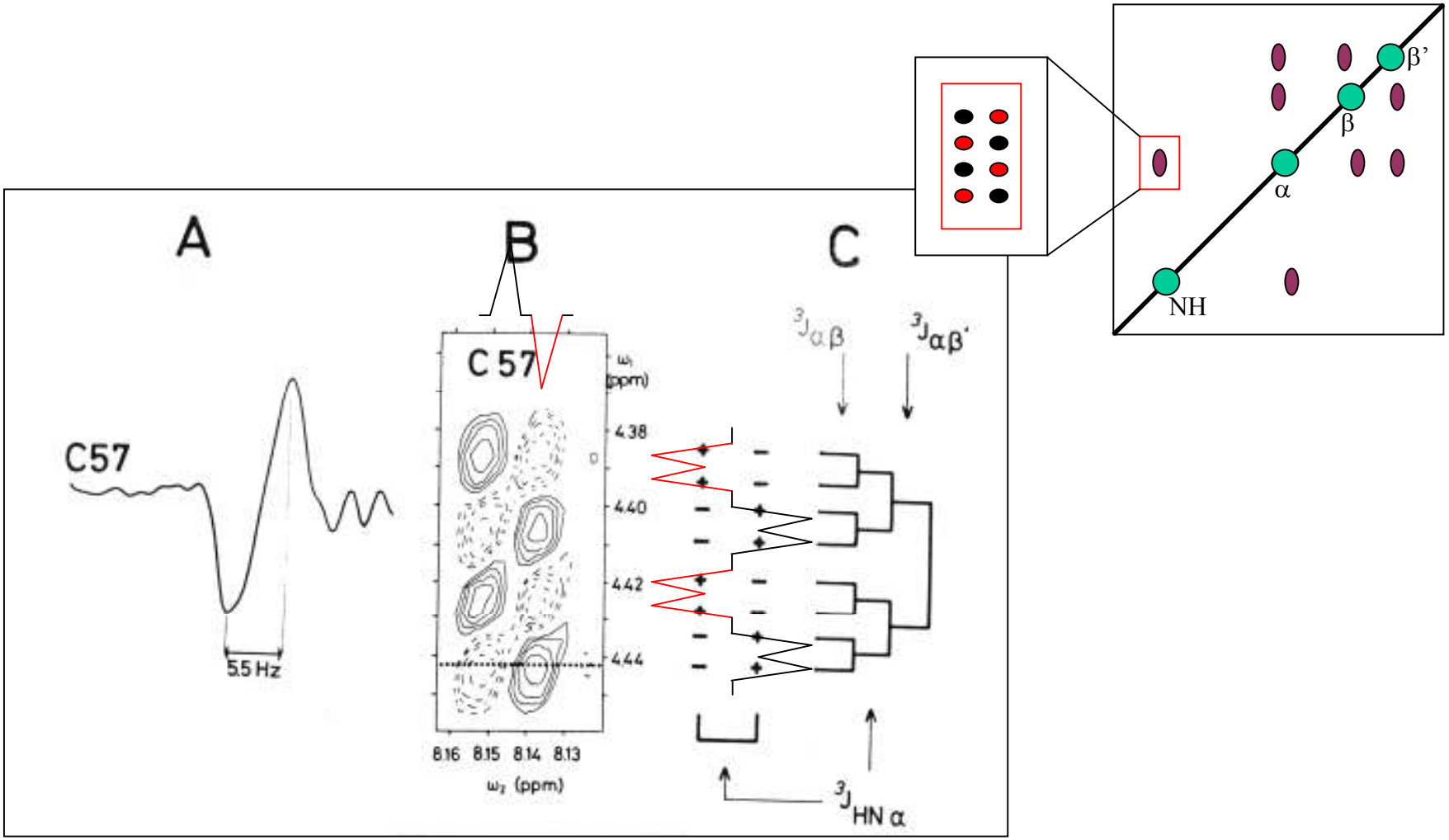


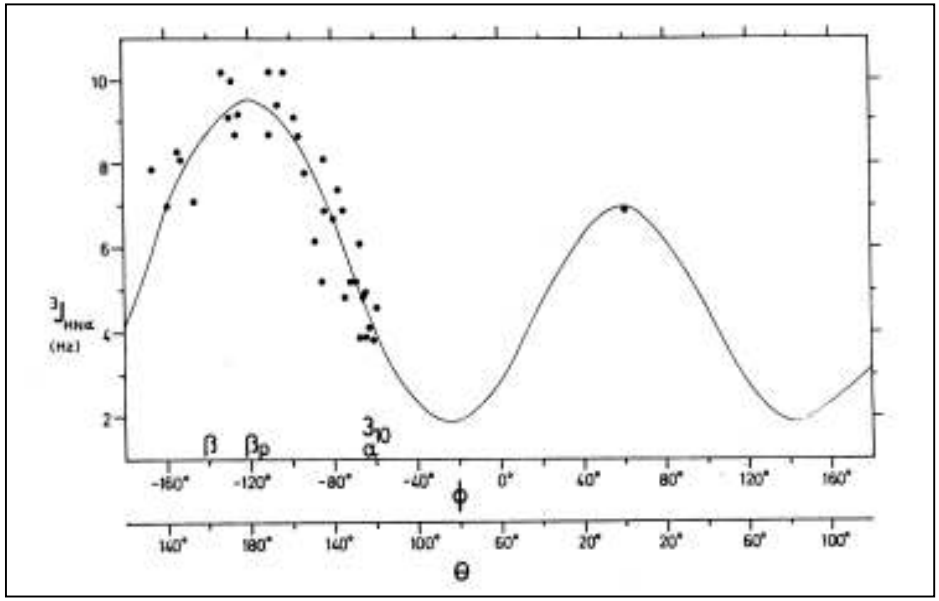
FIGURE 14. Overlap of adjacent orbiatals for ϕ=0° (left) and ϕ=90° (right).

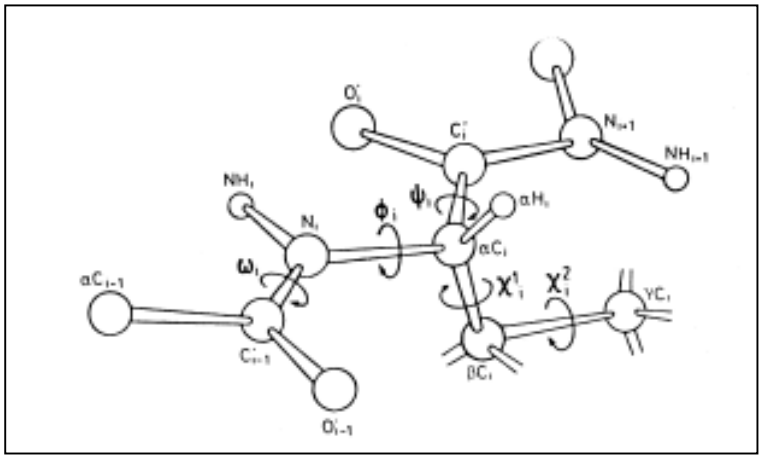
Scalar coupling

The analysis of the fine structure of protein 2D scalar correlations enables one to measure the ^3J couplings of αH with NH and the two βH.



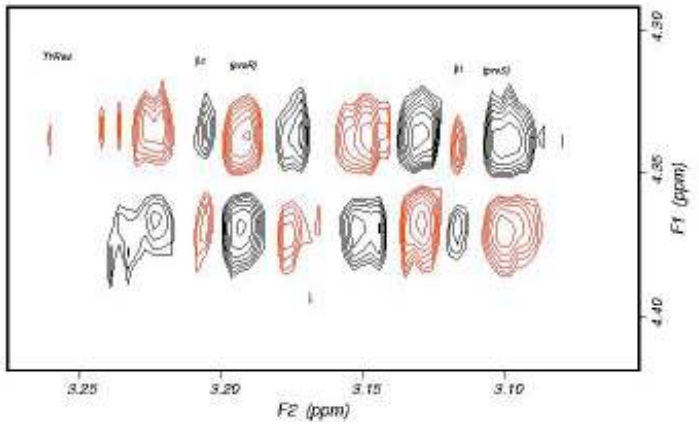
From the value of $J_{\mathrm{NH}\alpha}$, by means of a Karplus-type relationship, it is possible to estimate the peptide backbone dihedral angle ϕ.

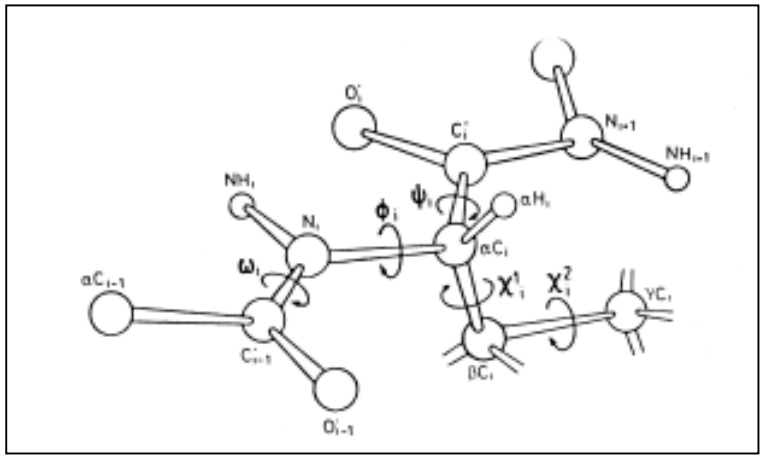


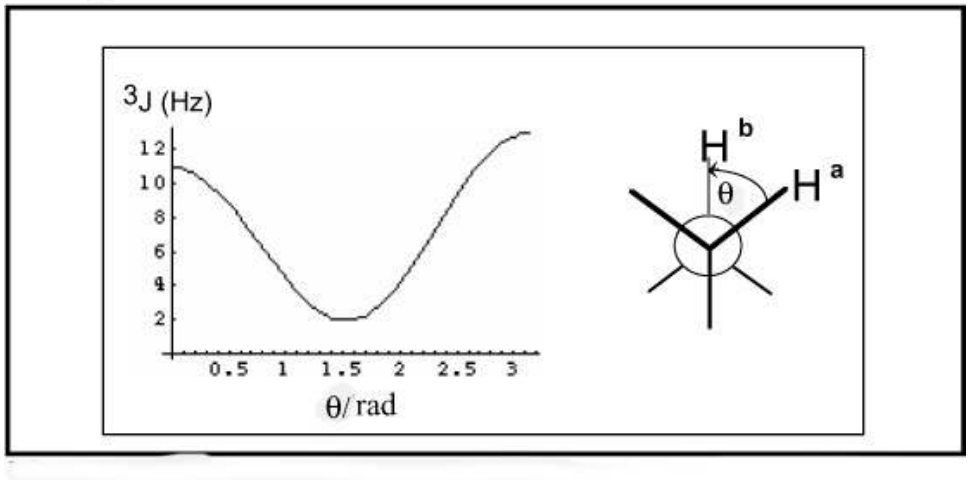


$$^3J_{NH\alpha} = 6.98\cos^2\theta - 1.38\cos\theta + 1.72$$

A similar analysis extended to $J_{\alpha\beta}$ coupling constants, enables one to extract information on the dihedral angle χ^1.

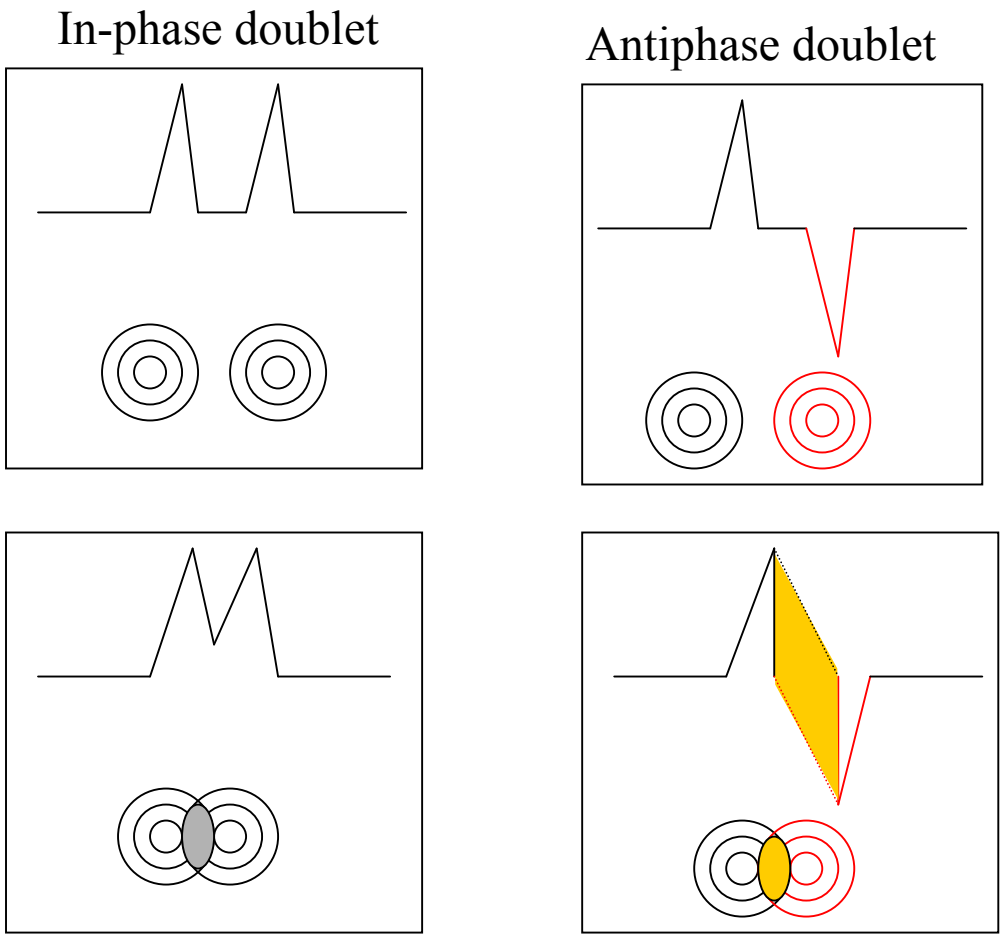






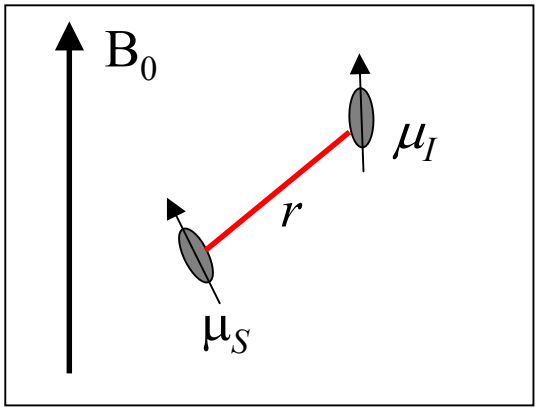
Scalar coupling

The measurement of the *J* coupling from peak spacing is hindered by limited experimental resolution and large linewidths

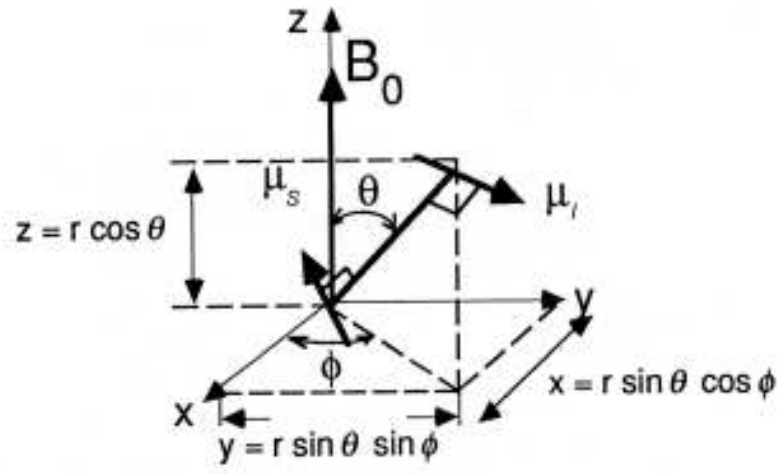


Dipolar interaction

The interaction between two nuclear magnetic dipoles, *I* and *S* (dipole-dipole interaction), generates a local field whose component in the direction of B_0 (z) affects the effective field at each nucleus by a factor:

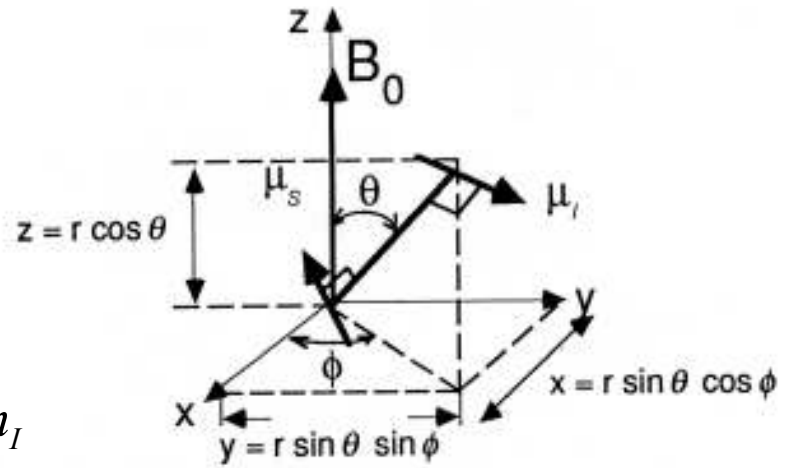


$$B_z^{dip} = \left(\frac{\mu_0}{4\pi}\right)\frac{\mu_z}{r^3}\left(3\cos^2\theta - 1\right)$$



Dipolar interaction

$$B_z^{dip} = \left(\frac{\mu_0}{4\pi}\right)\frac{\mu_z}{r^3}\left(3\cos^2\theta - 1\right)$$



$$\mu = \gamma \cdot J = \gamma\hbar\left[I\left(I+1\right)\right]^{\frac{1}{2}} \qquad \mu_z = \gamma \cdot J_z = \gamma\hbar m_I$$

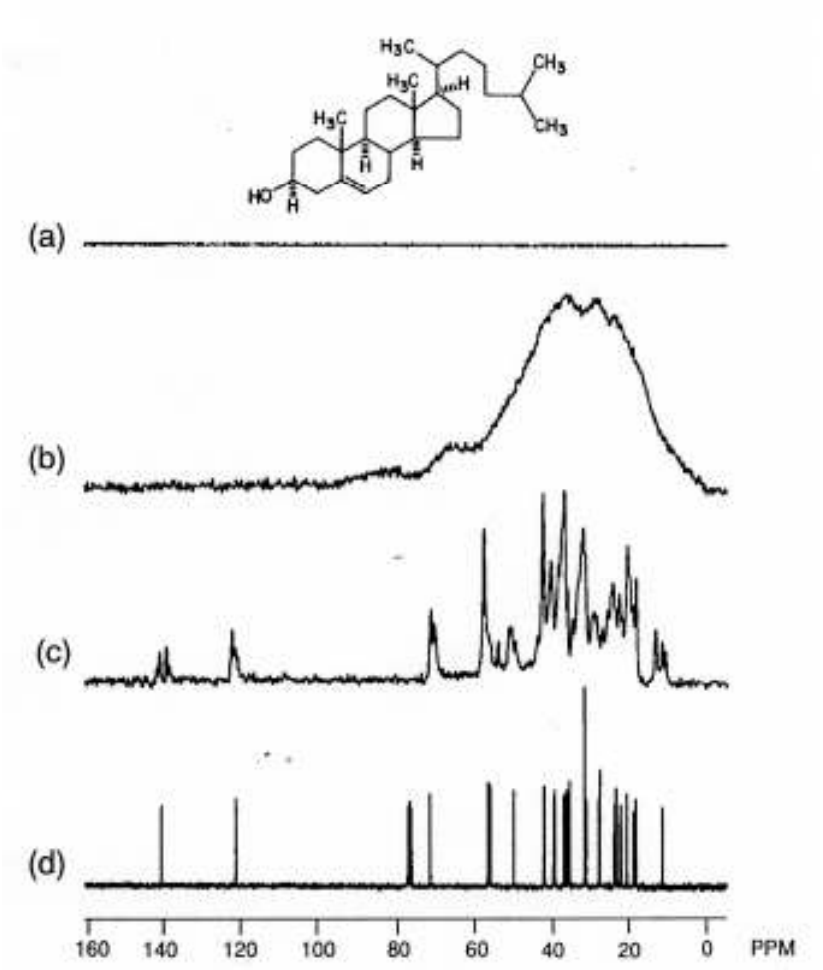
In practice, nucleus **S** changes its resonance frequency according to (2I+1) possible values of μ_z from nucleus **I**, whereas nucleus **I** changes its resonance frequency according to (2S+1) possible values of μ_z from nucleus **S**.

Dipolar coupling means resonance splitting; the dipolar coupling *D* depends on the external field.

Dipolar splittings are observed only in solids, either crystals or amorphous, or in liquid crystals.

Dipolar interaction

Solid state ^{13}C NMR spectrum of cholesterol (polycrystalline powder)



$$B_z^{dip} = \left(\frac{\mu_0}{4\pi}\right)\frac{\mu_z}{r^3}\left(3\cos^2\theta - 1\right)$$

a) basic solid state spectrum (severe broadening = a baseline)

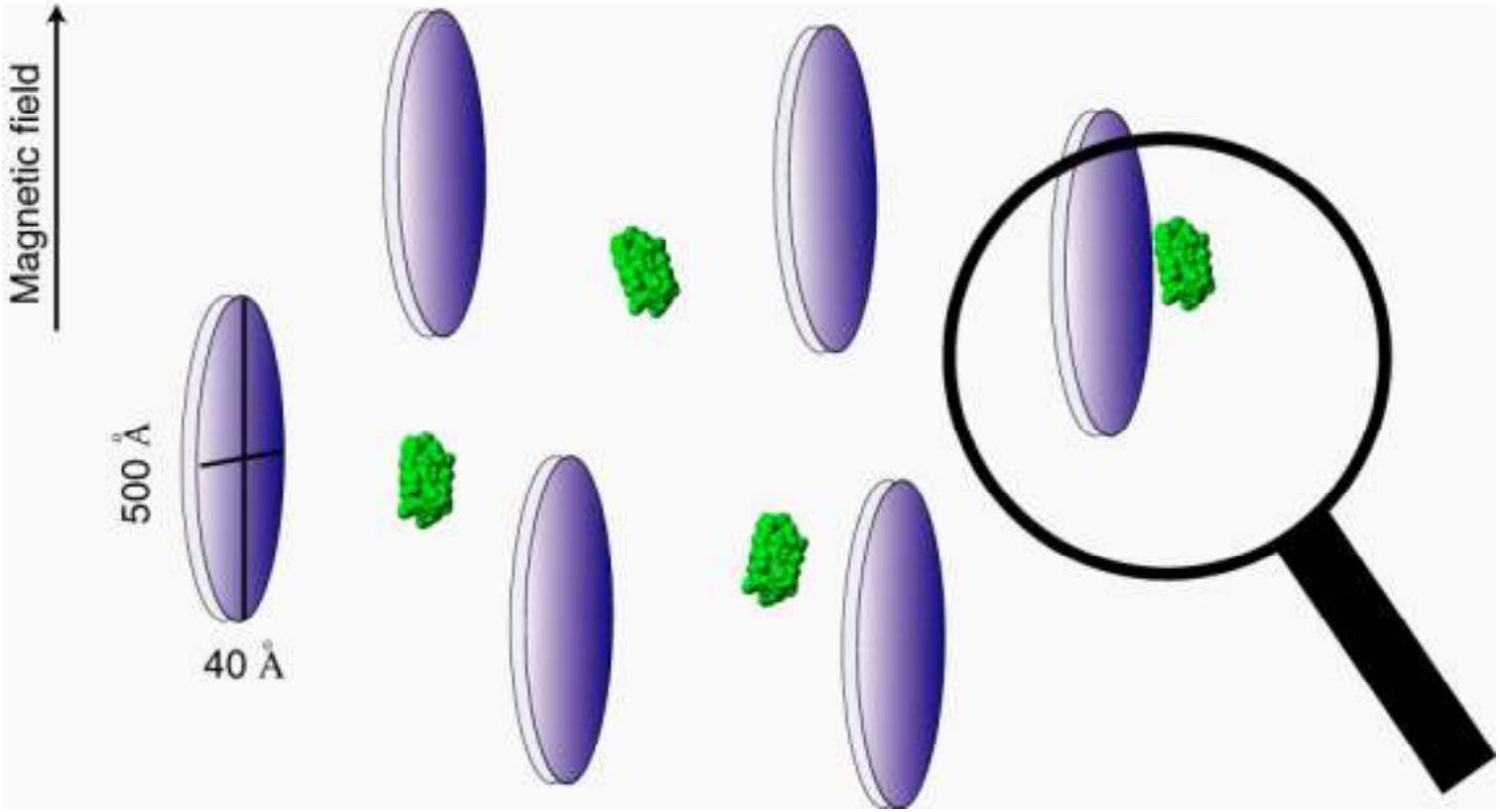
b) solid state spectrum with ^{1}H saturation to quench ^{1}H dipolar couplings (^{13}C dipolar couplings still present)

c) solid state spectrum in magic angle spinning mode: isotropic chemical shift are observed;

d) liquid state spectrum (in chloroform).

Dipolar interaction

Alignment by Liquid Crystal

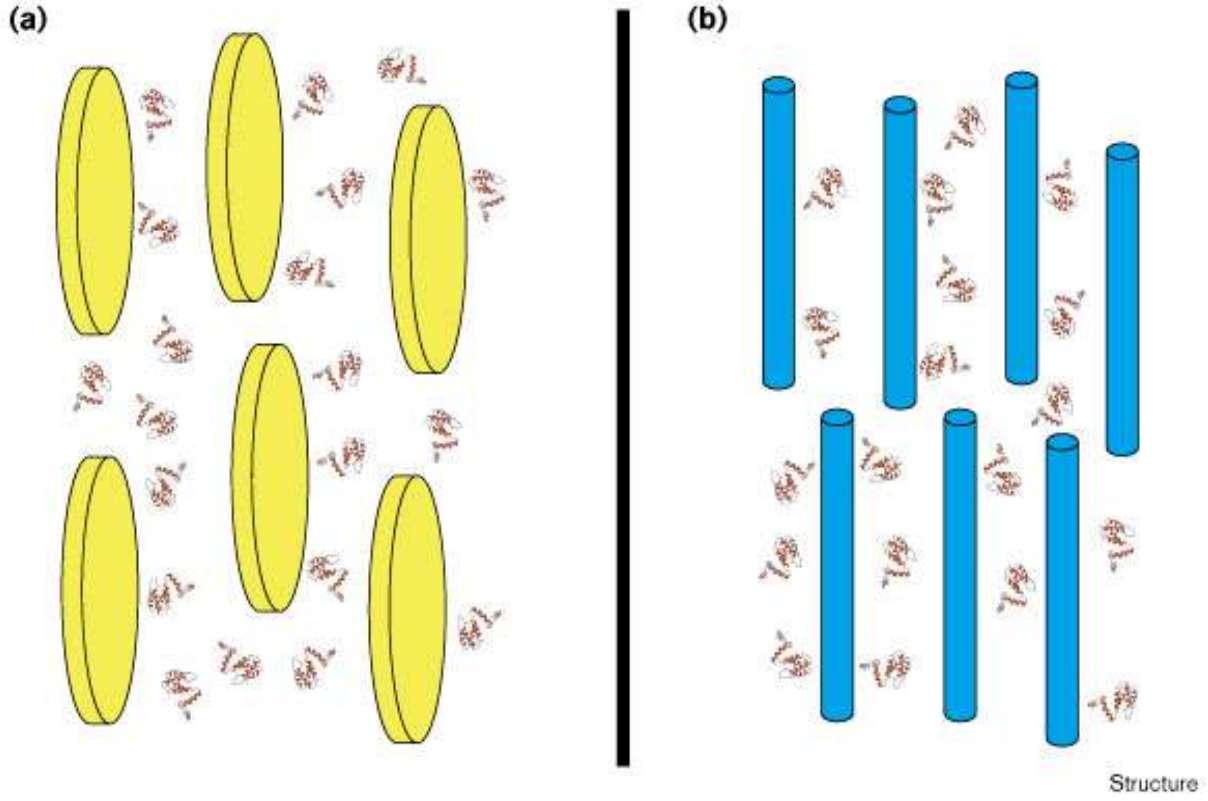


It is possible to restrict the motion of macromolecules in solution along preferential directions using proper orienting media.

Dipolar interaction

Dimyristoylphosphatidylcholine DMPC
Dihexanoylphosphatidylcholine DHPC

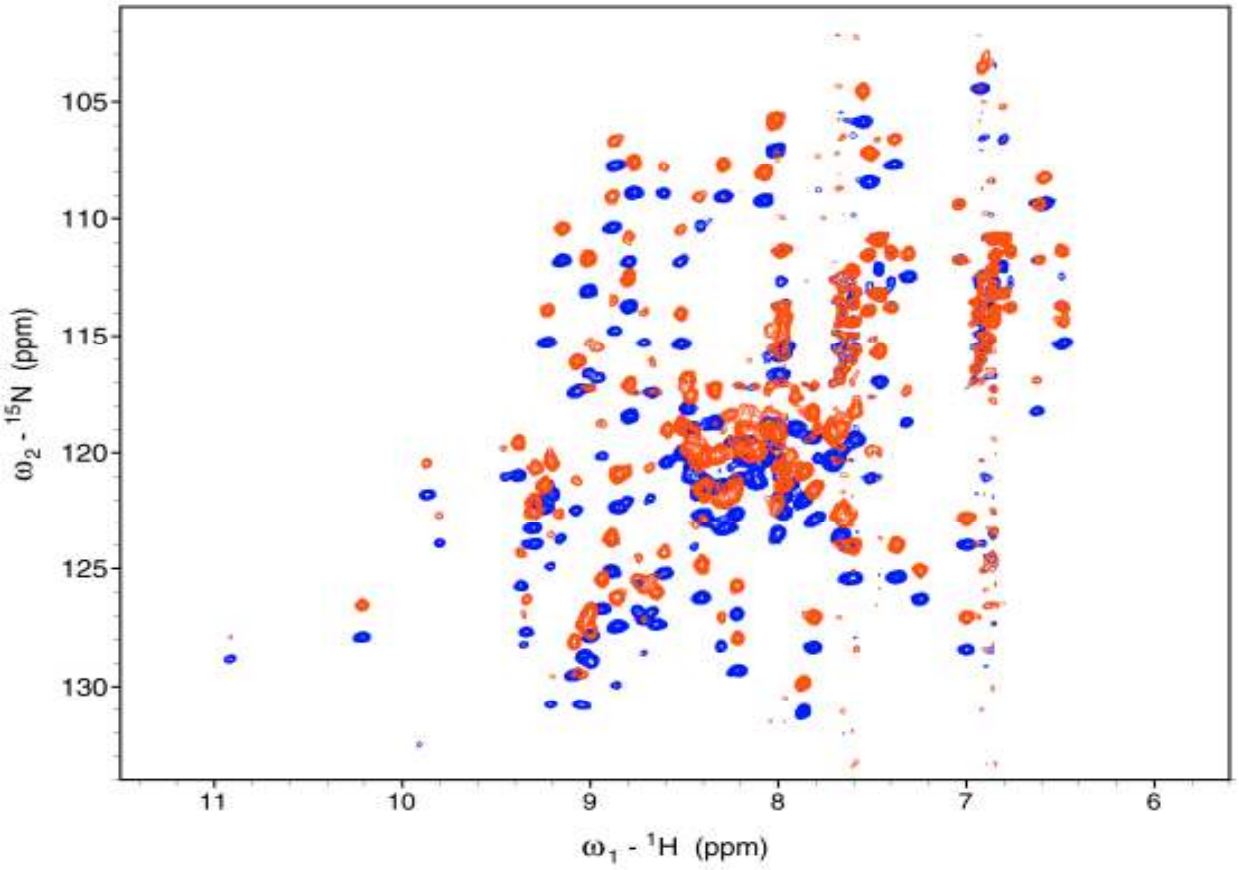
Filamentous Pf1 bacteriophage



Under these conditions it is possible to measure residual dipolar couplings, RDC.

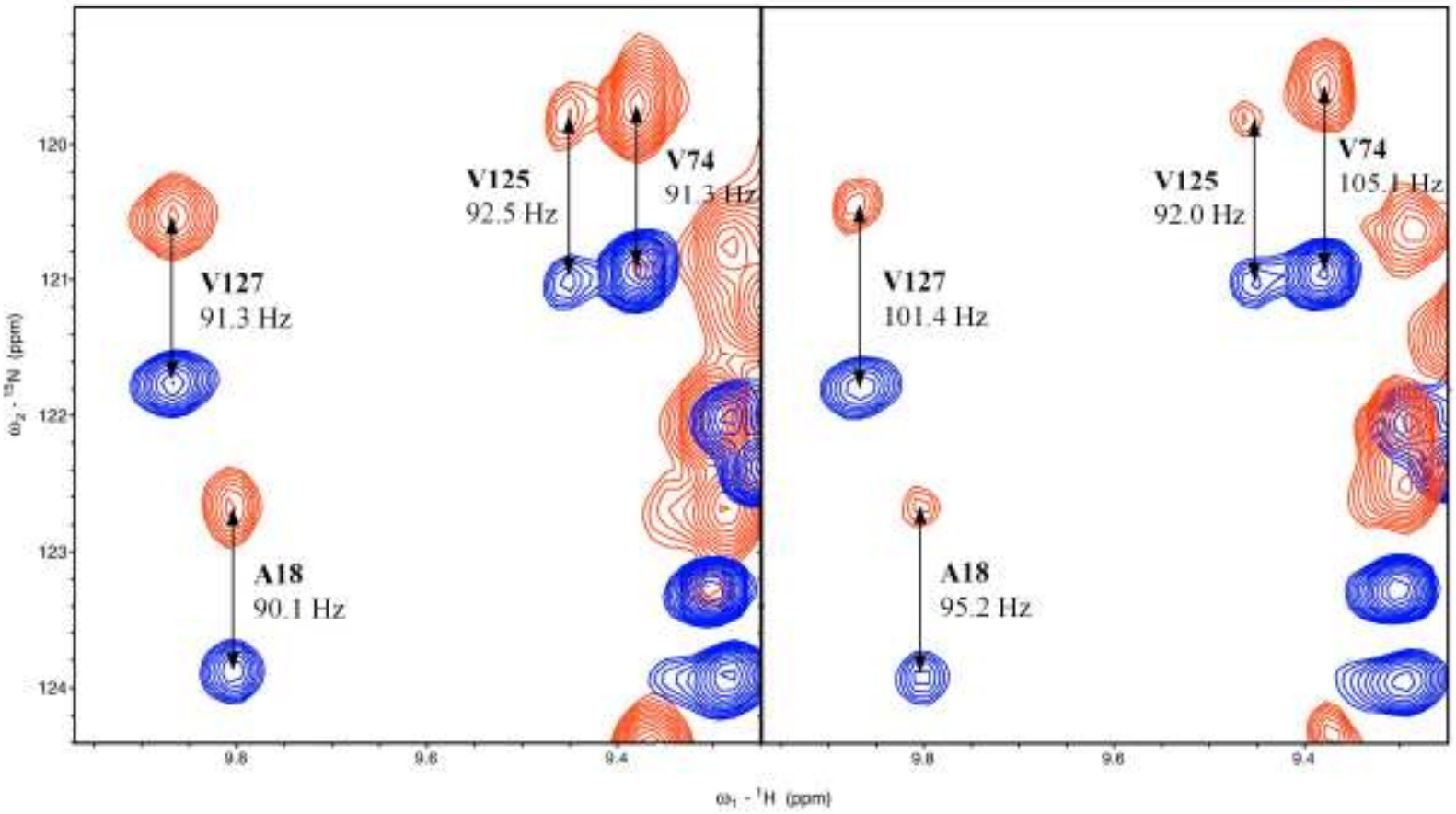
Dipolar interaction

RDC measurement in partially aligned media



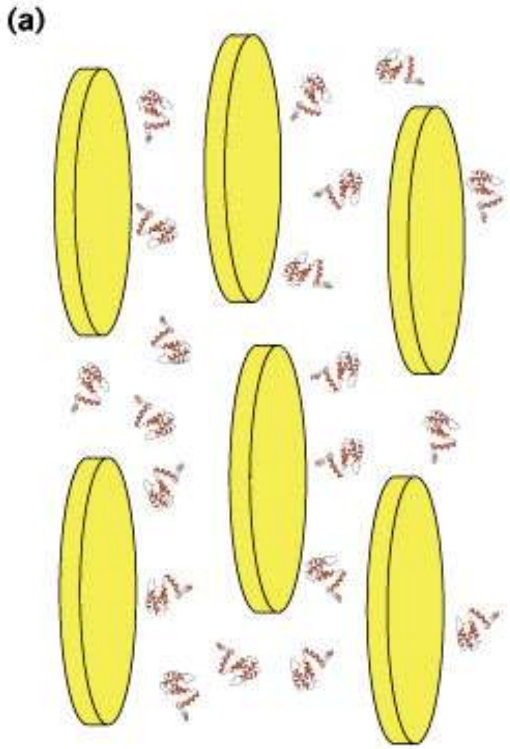
IPAP ^{1}H-^{15}N HSQC anisotropic solution

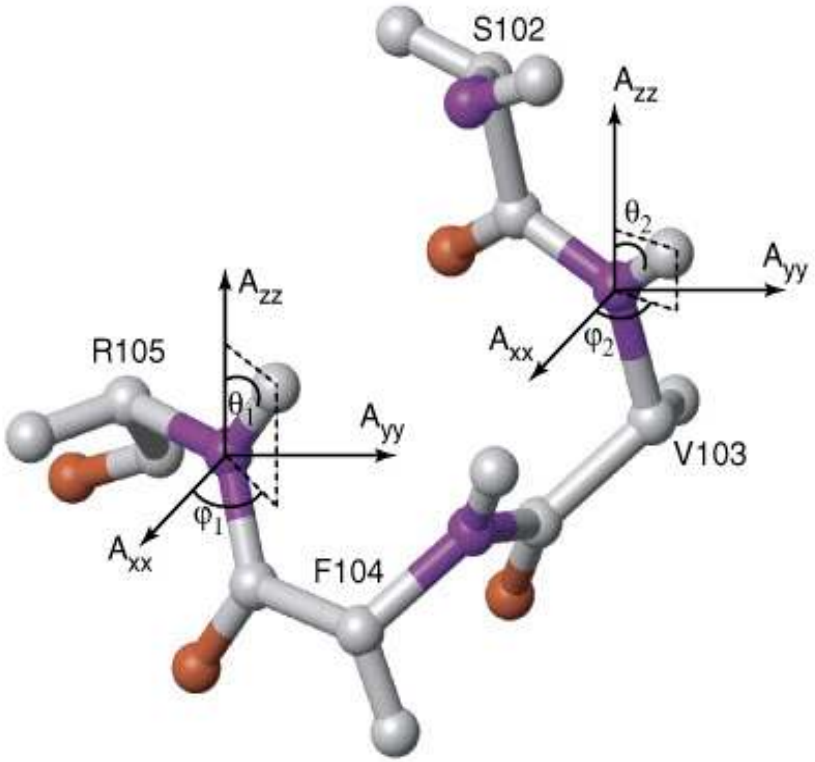
RDC measurement in partially aligned media



isotropic solution/anisotropic solution

RDC measurement in partially aligned media

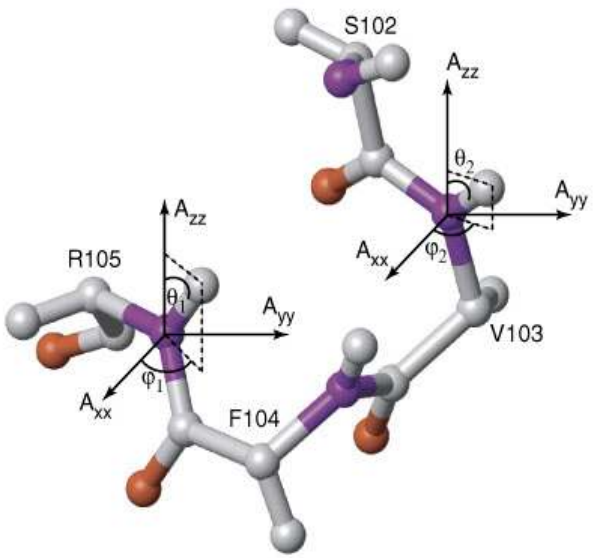




$$D_{HN(\theta,\phi)} = A_a^{HN}\left\{\left(3\cos^2\theta - 1\right) + \frac{3}{2}R\sin^2\theta\cos 2\varphi\right\}$$

Dipolar interaction

The RDC value of a nuclear pair depends on the orientation of the internuclear vector with respect to the overall molecular alignment tensor.



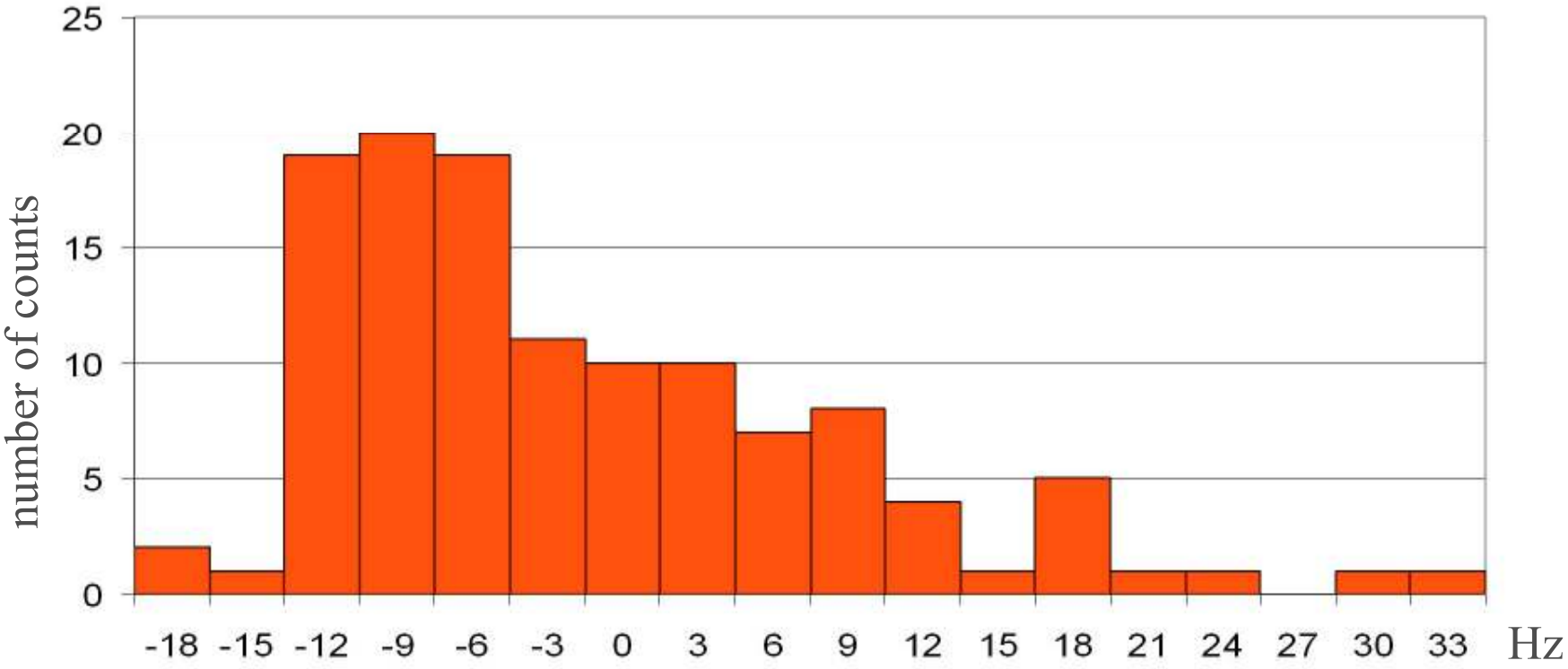
$$D_{AB(\theta,\phi)} = A_a^{AB}\left\{\left(3\cos^2\theta - 1\right) + \frac{3}{2}R\sin^2\theta\cos 2\varphi\right\}$$

A_a^{AB} axial, R rhombic components of the molecular alignment tensor, **A**, in the main reference frame. **A** components follow the rule $\left|A_{zz}\right| \geq \left|A_{yy}\right| \geq \left|A_{xx}\right|$

$$A_a = \frac{1}{3}\left[A_{zz}^{AB} - \left(\frac{A_{xx}^{AB} + A_{yy}^{AB}}{2}\right)\right] \quad A_r = \frac{1}{3}\left(A_{xx}^{AB} - A_{yy}^{AB}\right) \quad R = \frac{A_r}{A_a}$$

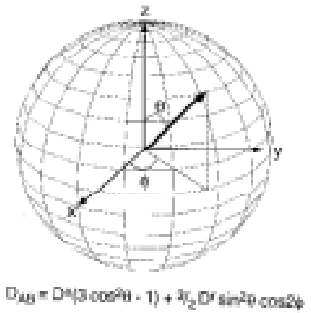
$$A_a^{AB} = -\frac{\mu_0 h}{16\pi^3} S\gamma_A\gamma_B r_{AB}^{-3} A_a$$

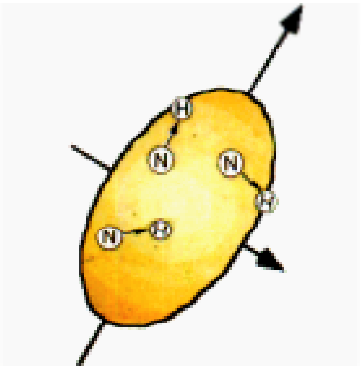
Distribution of 120 ^{1}H-^{15}N RDCs in C1q domain

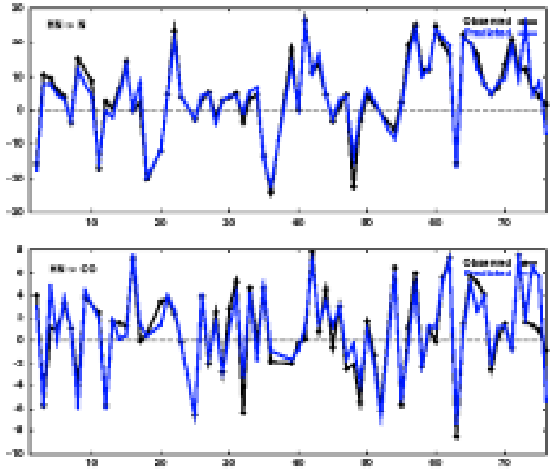


Dipolar interaction

RDC measurement in partially aligned media







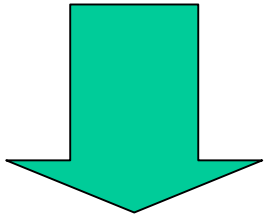
PALES is a software for handling RDCs

..... and in isotropic liquids?

$$B_z^{dip} = \left(\frac{\mu_0}{4\pi}\right)\frac{\mu_z}{r^3}\left(3\cos^2\theta - 1\right)$$

Molecular motions are rapid and effectively average, for all molecules, the factor $(3\cos^2\theta - 1)$. Globally, the contributions due to the longitidinal components of the local fields cancel out. For all nuclei:

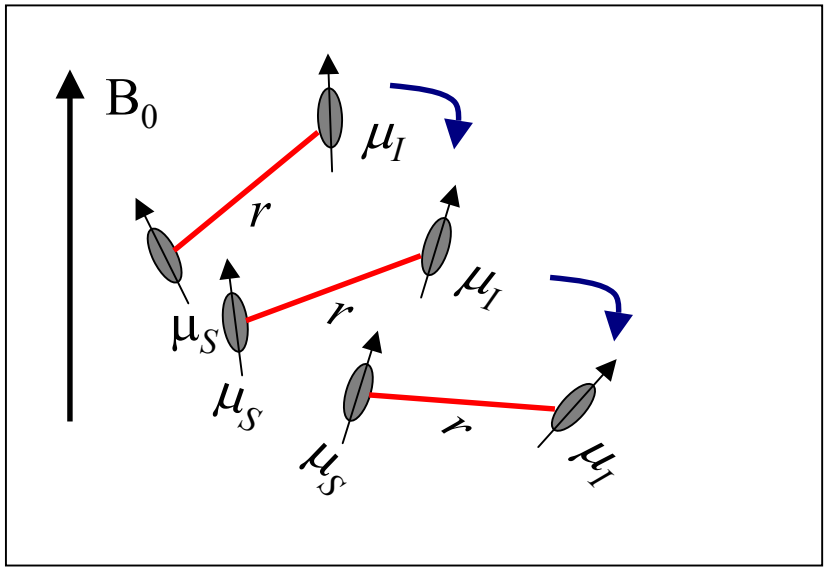
$$\left\langle B_z^{dip} \right\rangle = 0$$



This means that in isotropic liquids the dipolar interaction does not affect the chemical shift.

Dipolar relaxation

However, in isotropic liquids, the longitudinal and transverse components of B^{dip} are modulated by the molecular motion and thus generate fluctuating local magnetic fields.



When the fluctuation frequency has proper values, the variable local magnetic fields will be able to induce transitions, that is to cause relaxation:

$$\nu = \frac{\gamma}{2\pi} B_L$$

Dipolar relaxation mechanism

The event that ultimately determines NMR relaxation is the transition of a nuclear magnetic moment that occurs at a precise frequency value

$$\nu = \frac{\gamma}{2\pi} B_L$$

Dipolar relaxation

Consider the dipolar interaction between ^{1}H and ^{15}N of a protein amide.

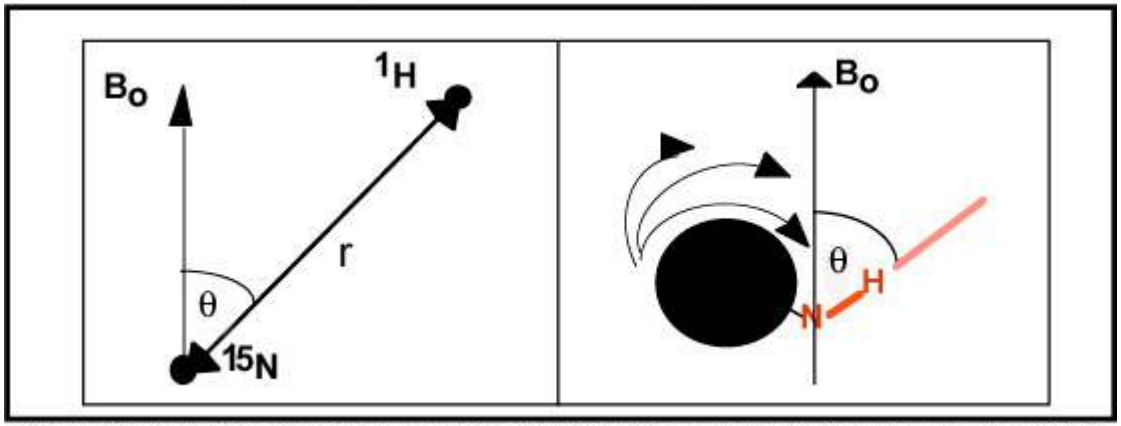


FIGURE 4. Left: definition of the vector connecting two spins and its orientation ϕ with respect to the external field. Right: Overall tumbling changes ϕ.

The reorientation rate of the internuclear vector determines a fluctuating local magnetic field, at each nucleus. This field depends on the distance between the nuclei and on their nature.

$$B_L \propto \left(\frac{\mu_0}{4\pi}\right)\frac{\mu}{r_{IS}^3} \qquad \mu_I = \gamma_I \hbar\left[I(I+1)\right]^{\frac{1}{2}} \qquad \mu_S = \gamma_S \hbar\left[S(S+1)\right]^{\frac{1}{2}}$$

Dipolar relaxation

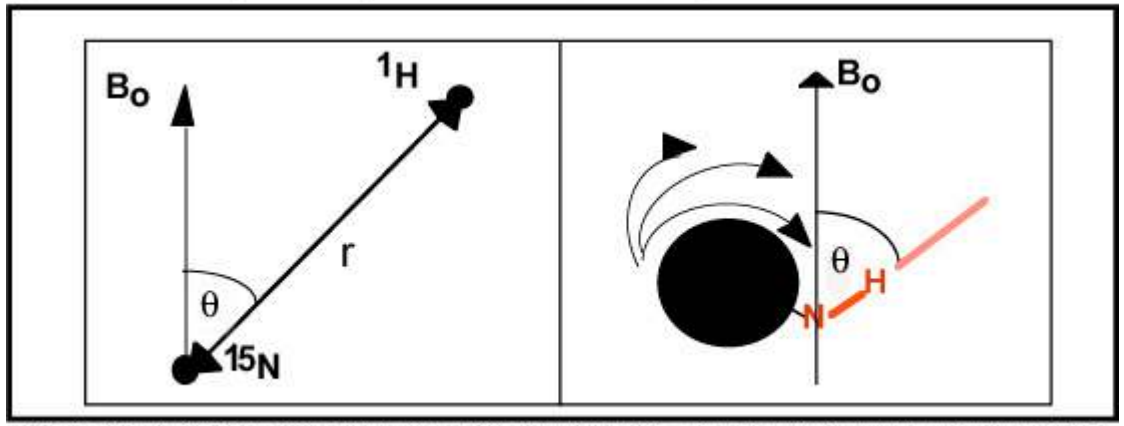


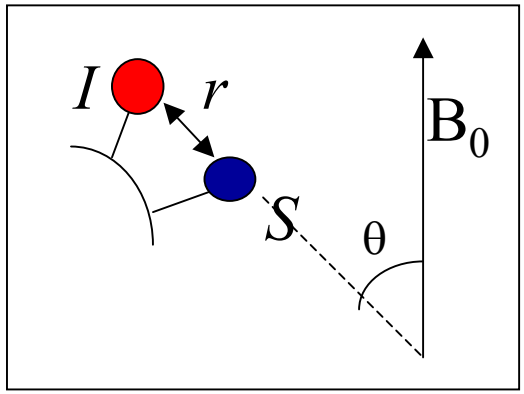
FIGURE 4. Left: definition of the vector connecting two spins and its orientation ϕ with respect to the external field. Right: Overall tumbling changes ϕ.

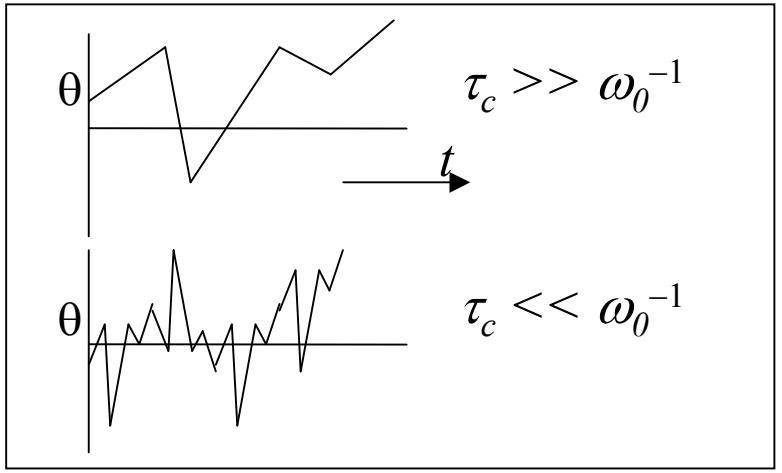
Therefore, the overall effect of the dipolar mechanism on relaxation is an increased transition probability (W) proportional to:

$$W \propto \left[\left(\frac{\mu_0}{4\pi}\right)\frac{\gamma_I \gamma_S \hbar}{r_{IS}^3}\right]^2 \Rightarrow \propto r_{IS}^{-6}$$

We can measure the dipolar relaxation extent within pairs of nuclei.

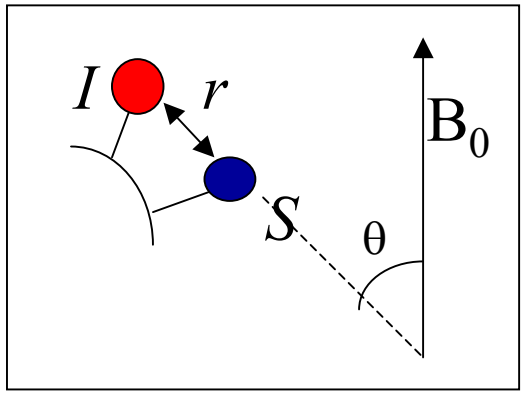
Cross-relaxation, Nuclear Overhauser Effect - NOE

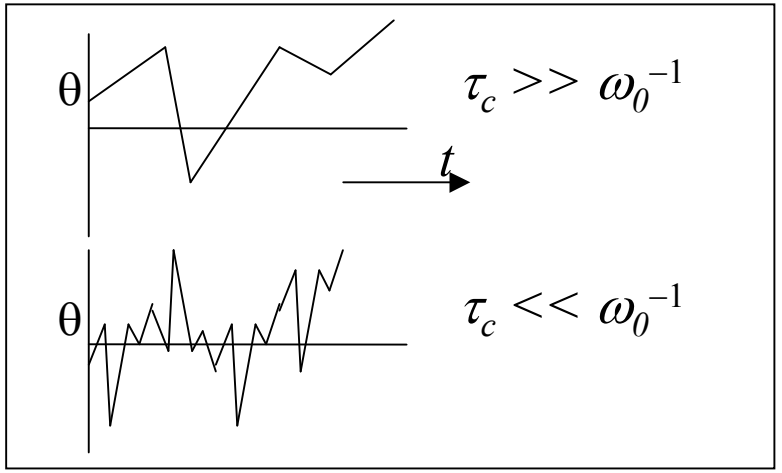




The extent of dipolar relaxation between two nuclei in a static external field depends on the interatomic separation and on the rate of reorientation of the internuclear vector, the frequency of θ change, with respect to the reciprocal of Larmor frequency, i.e with respect to the external magnetic.

Cross-relaxation, Nuclear Overhauser Effect - NOE





We can measure NOE (η) that is a function of cross relaxation (σ), in turn related to internuclear distance and internuclear vector reorientation (molecular motion):

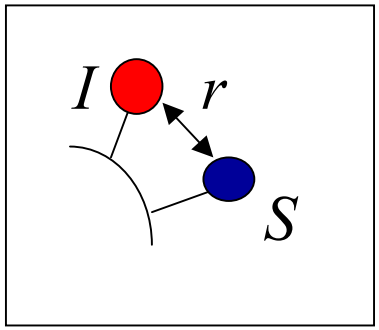
$$\eta = f(\sigma) = f\left(\tau_c, r_{IS}^{-6}\right)$$

$$\sigma_{ij} = \frac{\hbar^2\gamma^2}{10 r_{ij}^6}\left[\frac{6\tau_c}{1+4\omega^2\tau_c^2} - \tau_c\right]$$

cross-relaxation rate

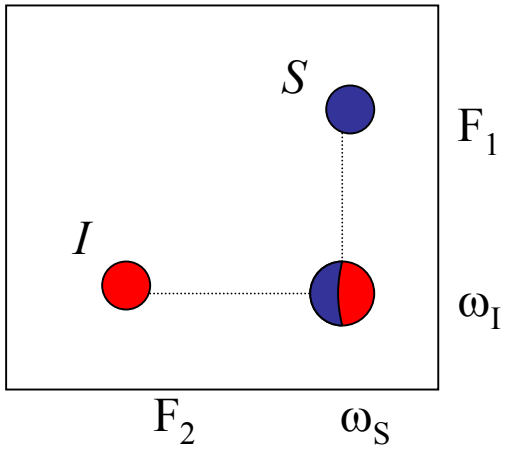
Cross-relaxation, Nuclear Overhauser Effect - NOE

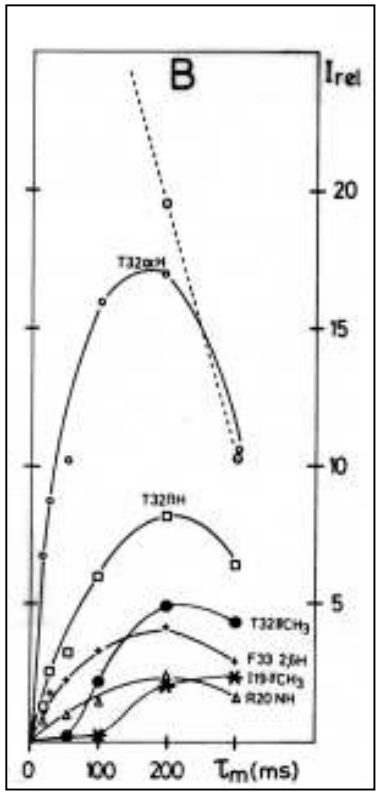
NOE can be measured from NOESY experiments



$$\text{NOE} = f\left(\tau_c, r_{IS}^{-6}\right)$$

$$\text{cp}_{\text{NOESY}} = f\left(\tau_c, r_{IS}^{-6}, t_m\right)$$

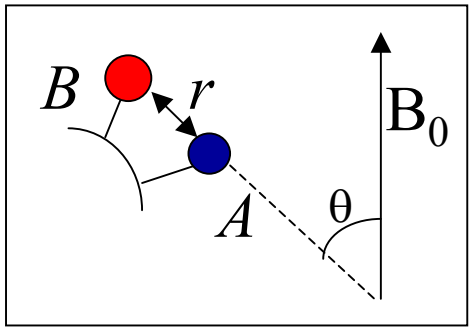


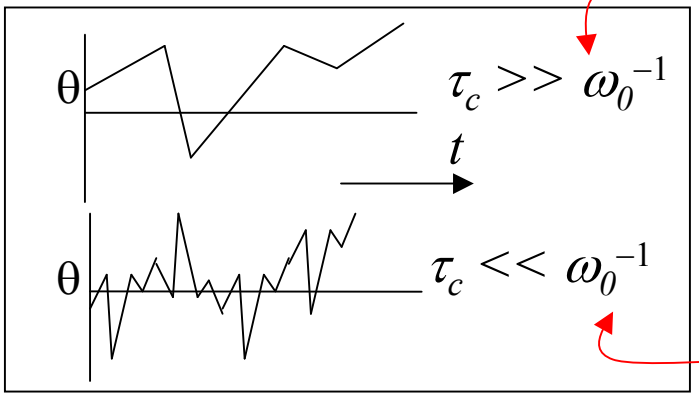


The intensity of a NOESY cross-peak depends on the NOE and the experimental *mixing time*, t_m .

Cross-relaxation, Nuclear Overhauser Effect - NOE

NOESY cp and molecular mobility





Negative NOE

spin diffusion limit

Positive NOE

extreme narrowing limit

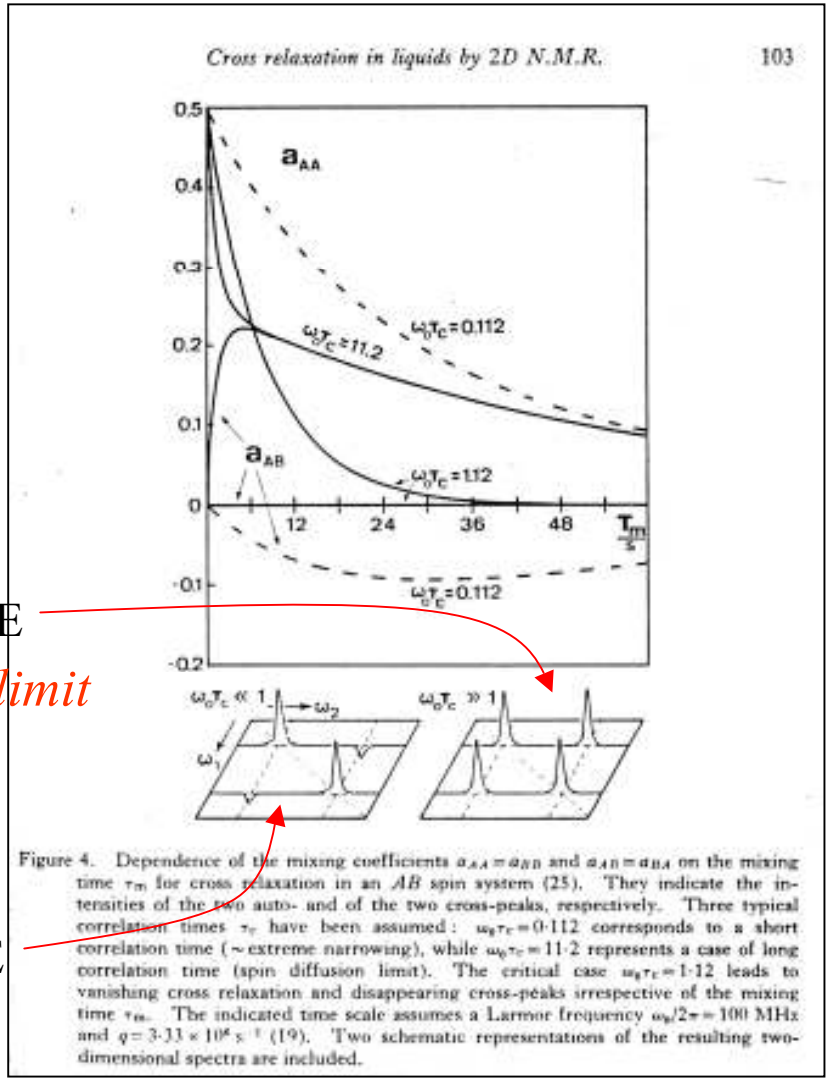
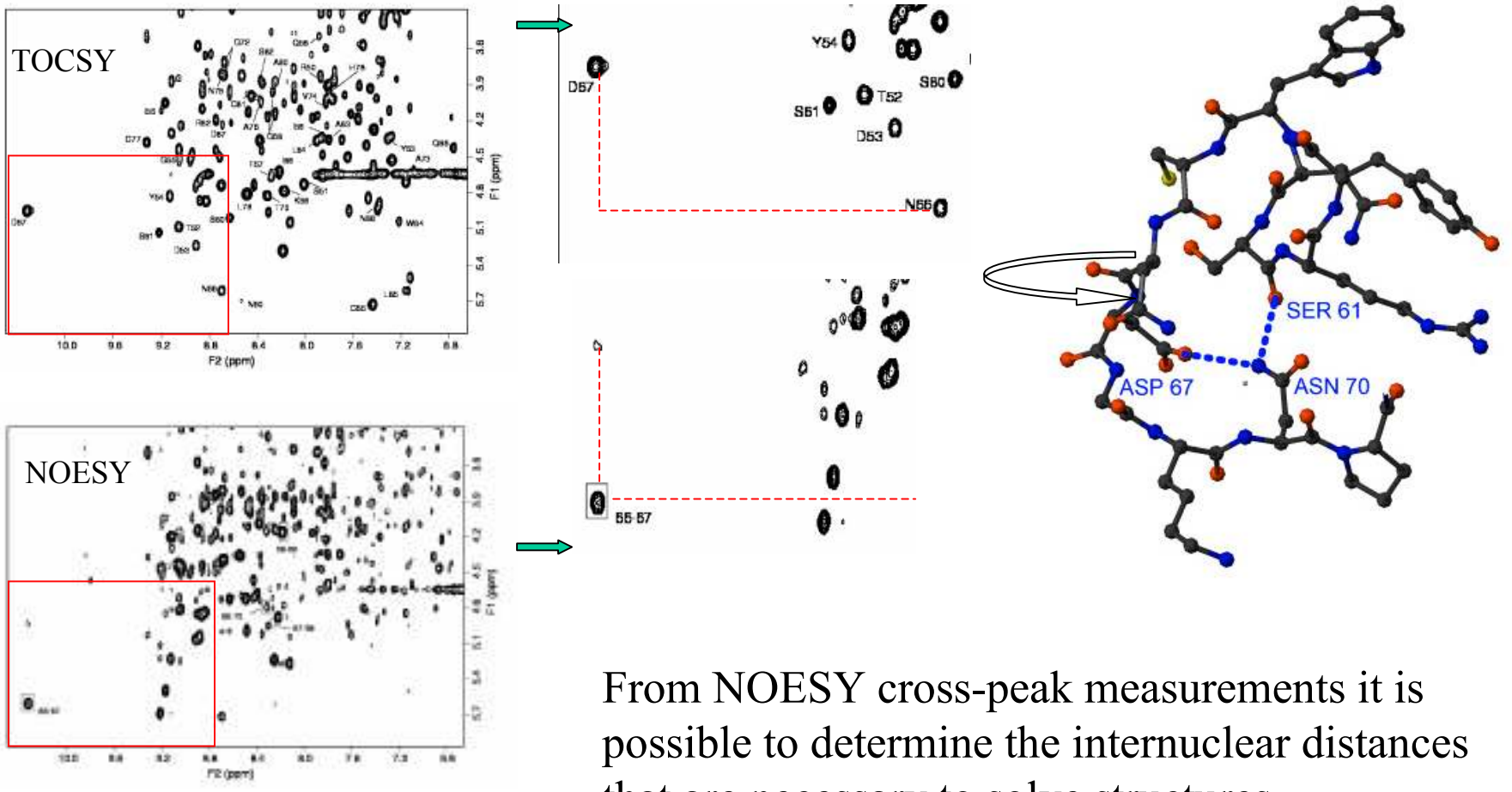


Figure 4. Dependence of the mixing coefficients $a_{AA} = a_{BB}$ and $a_{AB} = a_{BA}$ on the mixing time τ_m for cross relaxation in an AB spin system (25). They indicate the intensities of the two auto- and of the two cross-peaks, respectively. Three typical correlation times τ_c have been assumed: $\omega_0\tau_c = 0{\cdot}112$ corresponds to a short correlation time (~extreme narrowing), while $\omega_0\tau_c = 11{\cdot}2$ represents a case of long correlation time (spin diffusion limit). The critical case $\omega_0\tau_c = 1{\cdot}12$ leads to vanishing cross relaxation and disappearing cross-peaks irrespective of the mixing time τ_m. The indicated time scale assumes a Larmor frequency $\omega_0/2\pi = 100$ MHz and $q = 3{\cdot}33 \times 10^8\ s^{-1}$ (19). Two schematic representations of the resulting two-dimensional spectra are included.

Macura & Ernst, *Mol. Phys.*, 1980

Cross-relaxation, Nuclear Overhauser Effect - NOE



From NOESY cross-peak measurements it is possible to determine the internuclear distances that are necessary to solve structures.

Cross-relaxation,
Nuclear Overhauser Effect - NOE

From NOESY cross-peak measurements it is possible to determine the internuclear distances that are necessary to solve structures.

Table 2. *Structure validation parameters for human β2-m conformers from NMR data refinement (DISCOVER)*

Parameter	This work	Consensus standard[a]
Total residues/selected residues[a]	100/83	74 ± 39/62 ± 36
Number of conformers	20	20 ± 15
Restraints per residue[c,d]	10.3	11.3 ± 4.5
NOE rms violation ($\times 10^{-1}$ nm)[e]	0.079	0.061 ± 0.043
Ramachandran quality[b]		
Residues in most-favored regions	70.5%	73.6 ± 15.5%
Residues in additional allowed regions	28.2%	n.a.
Residues in generously allowed regions	1.0%	n.a.
Residues in disallowed regions	0.3%	n.a.
Average G-factors[b,f]		
$\varphi - \psi$	−1.13	n.a.
$\chi^1 - \chi^2$	−1.03	n.a.
χ^1 only	0.14	n.a.
overall	−0.98	n.a.