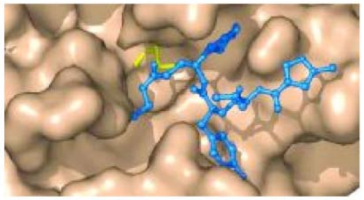
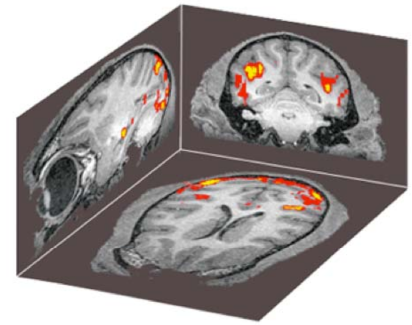
NMR Spectroscopy

Applications

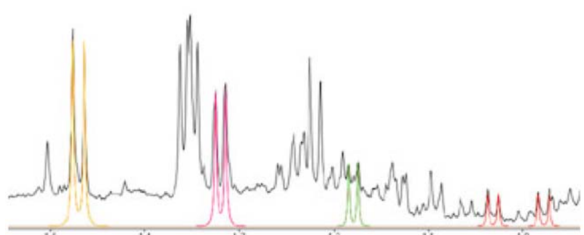


Drug design



MRI

Food quality



Metabonomics

Structural biology

NMR Spectroscopy

Basic Principles

N.M.R. = Nuclear Magnetic Resonance

Spectroscopic technique, thus relies on the interaction between material and electromagnetic radiation

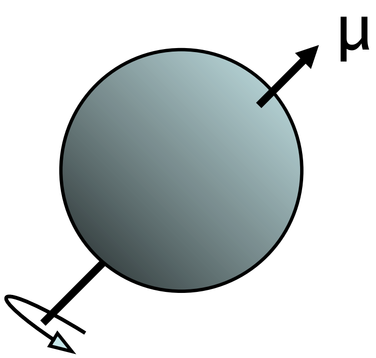
The nuclei of all atoms possess a nuclear quantum number, **I**. ($I \geq 0$, always multiples of ½.)

Only nuclei with spin number (I) >0 can absorb/emit electromagnetic radiation.

Even atomic mass & number: I = 0 (^{12}C, ^{16}O)

Even atomic mass & odd number: I = whole integer (^{14}N, ^{2}H, ^{10}B)

Odd atomic mass: I = half integer (^{1}H, ^{13}C, ^{15}N, ^{31}P)



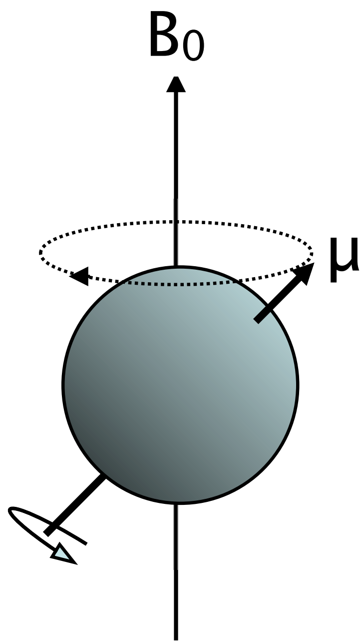
The spinning nuclei possess angular momentum, P, and charge, and so an associated magnetic moment, μ.

$$\mu = \gamma \times P$$

Where γ is the gyromagnetic ratio

NMR Spectroscopy

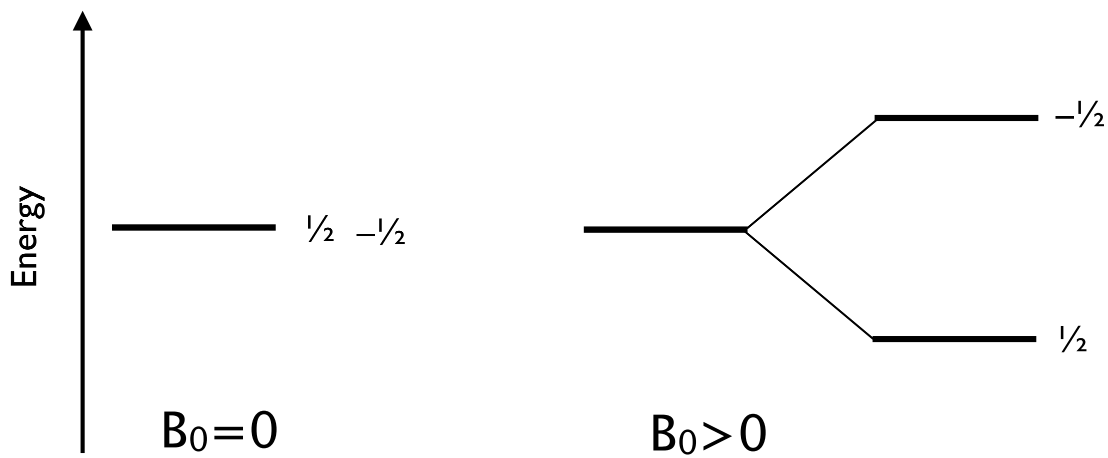
Basic Principles



The spin states of the nucleus are quantified:

I, (I - 1), (I - 2), … , -I

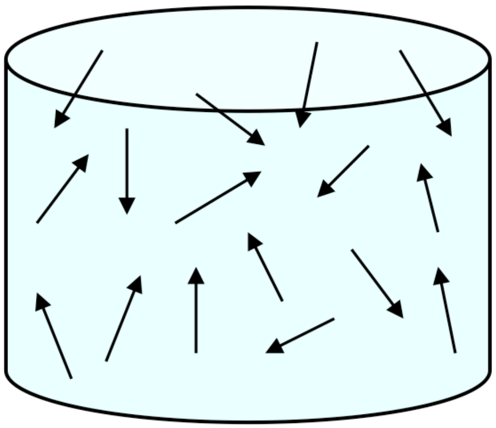
I= ½ (e.g. [1]H)



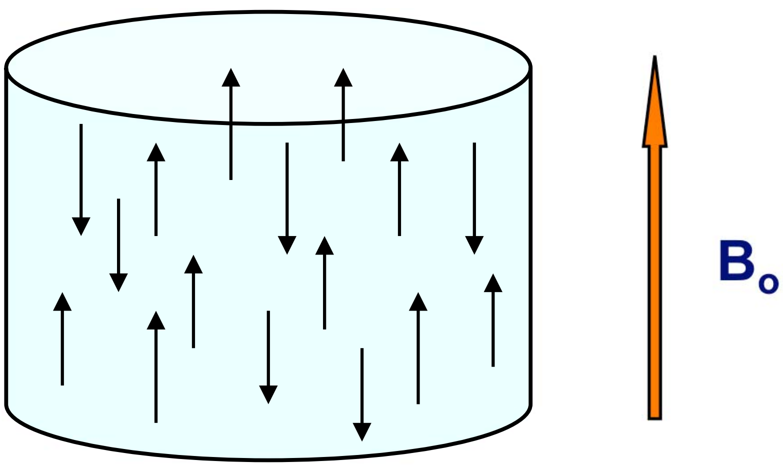
$$\Delta E = h\nu = h\gamma B_0/2\pi$$

NMR Spectroscopy

Basic Principles



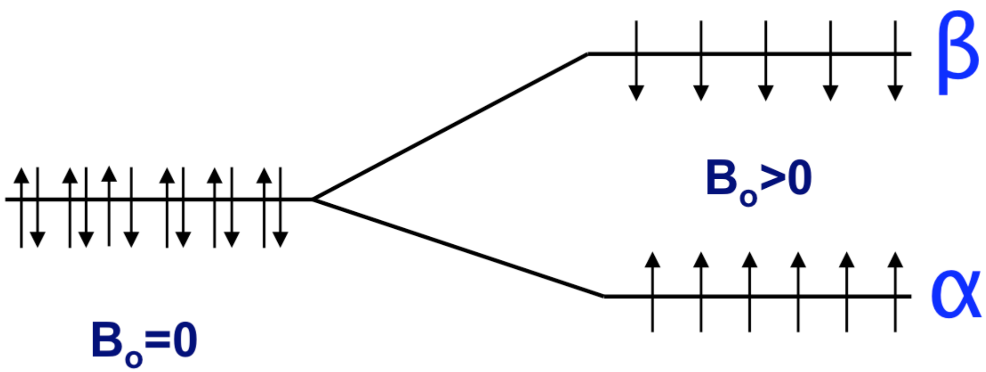
In the ground state all nuclear spins are disordered, and there is no energy difference between them. They are degenerate.



Since they have a magnetic moment, when we apply a strong external magnetic field (Bo), they orient either against or with it:
There is always a small excess of nuclei (population excess) aligned with the field than pointing against it.

NMR Spectroscopy

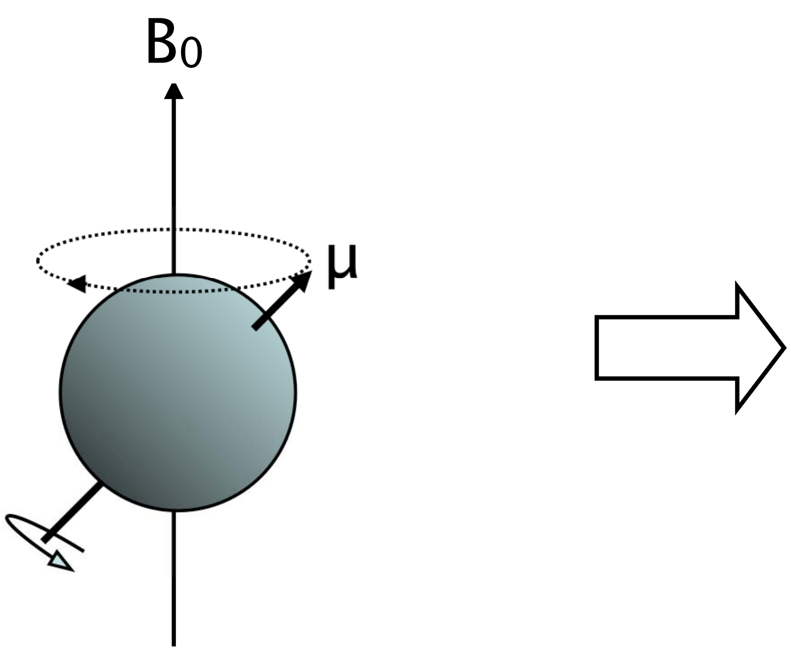
Basic Principles

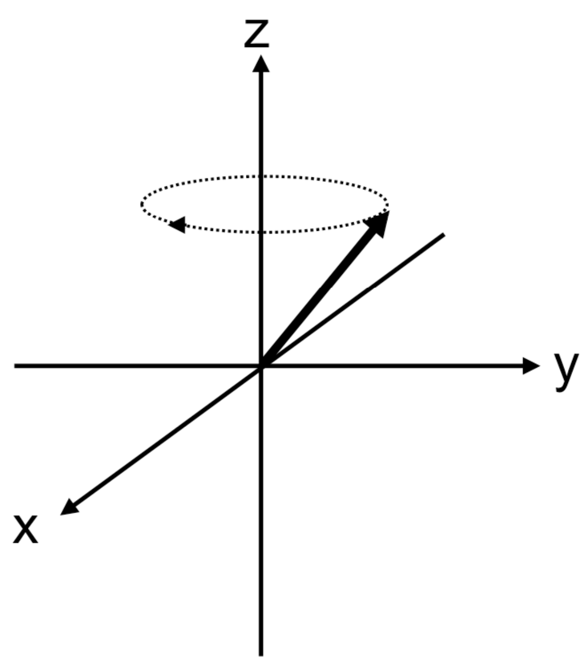


$\Delta E = h\nu_0 = h\gamma B_0/2\pi$

ν_0 is the Larmor Frequency

$\omega_0 = \gamma B_0$, angular velocity





NMR Spectroscopy

Basic Principles

Each level has a different population (N), and the difference between the two is related to the energy difference by the Boltzmman distribution:

$$N_\alpha / N_\beta = e^{\Delta E / kT}$$

ΔE for ^{1}H at 400 MHz (B_0 = 9.5 T) is 3.8×10^{-5} Kcal/mol

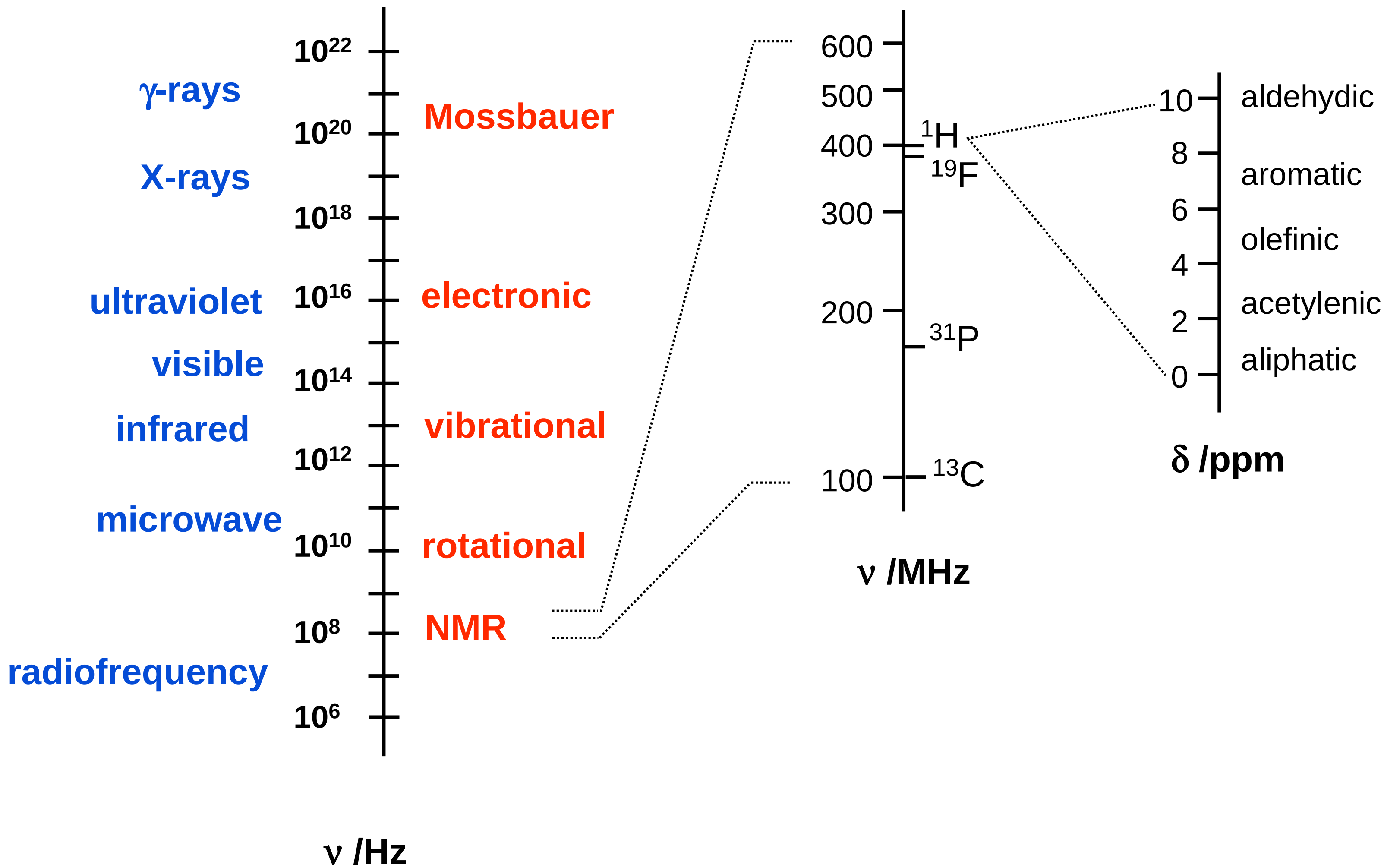
$$N_\alpha / N_\beta = 1.000064$$

The surplus population is small (especially when compared to UV or IR).

That renders NMR a rather insensitive technique!

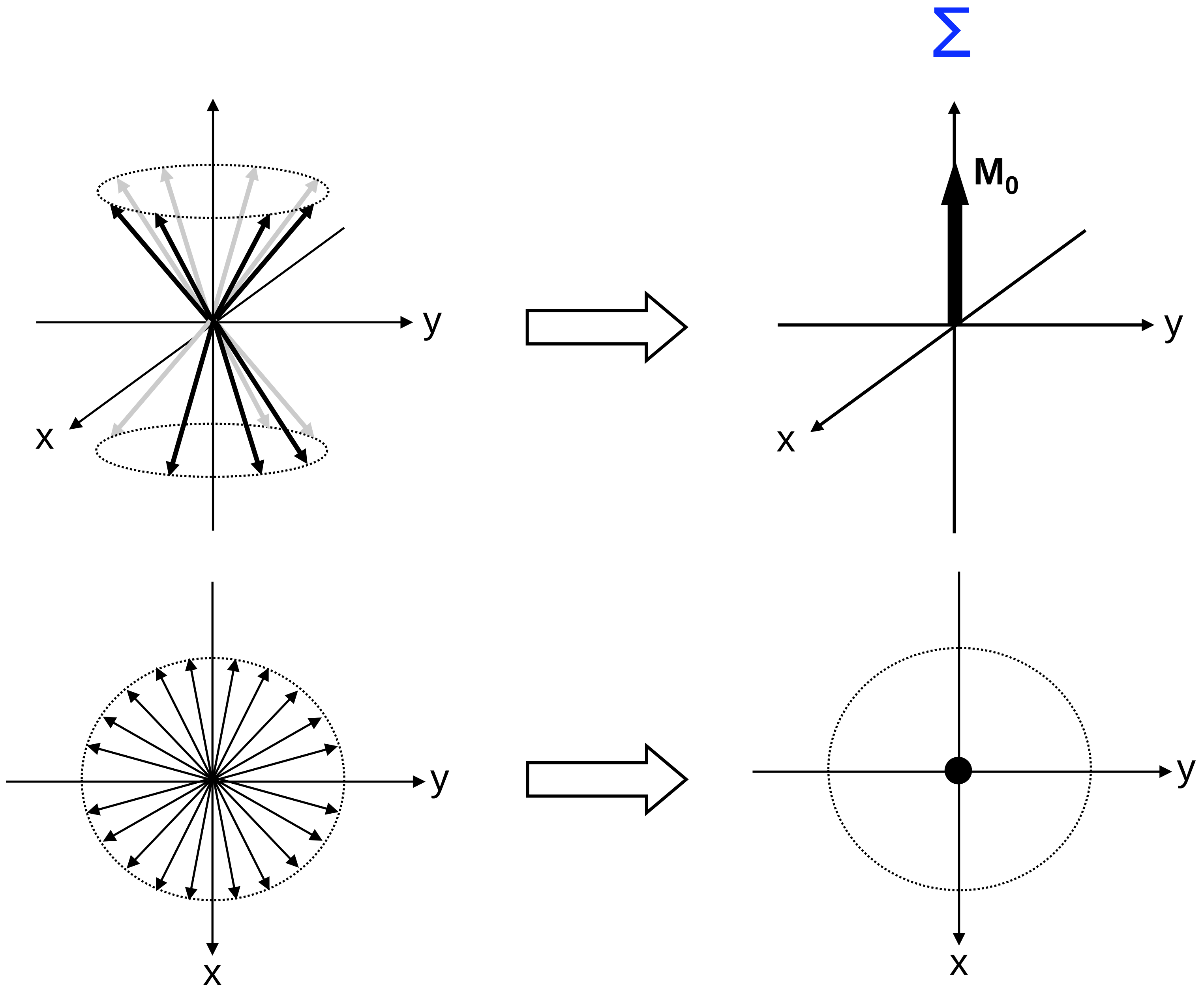
NMR Spectroscopy

The electromagnetic spectrum



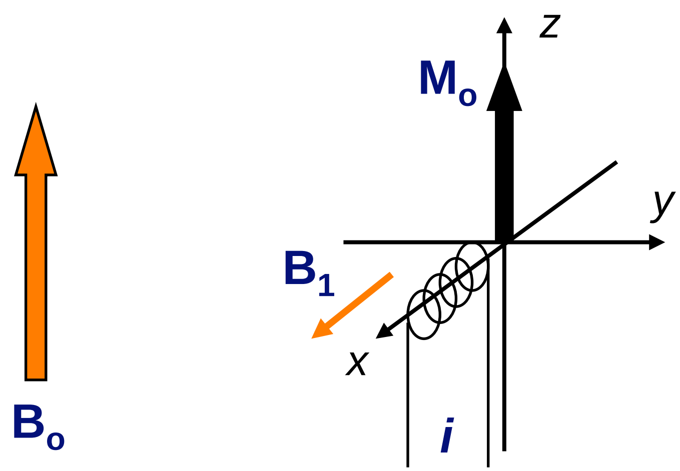
NMR Spectroscopy

The Vector Model

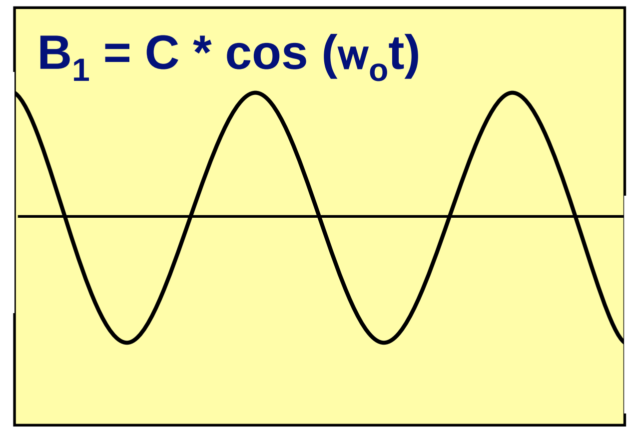


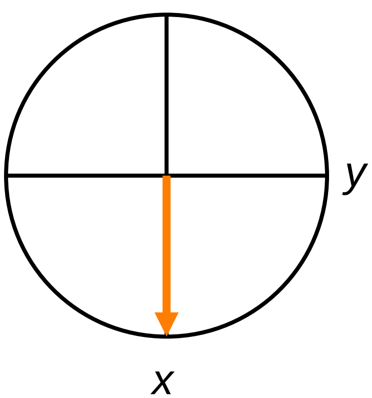
NMR Spectroscopy

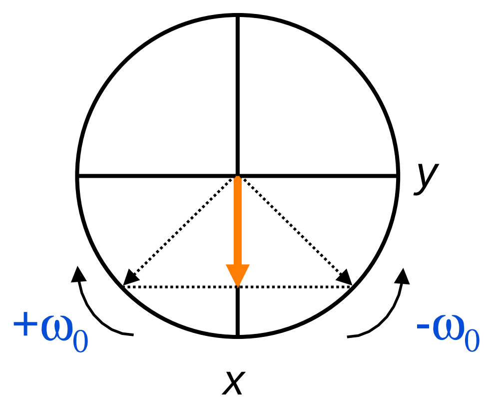
NMR excitation



Transmitter coil (x)



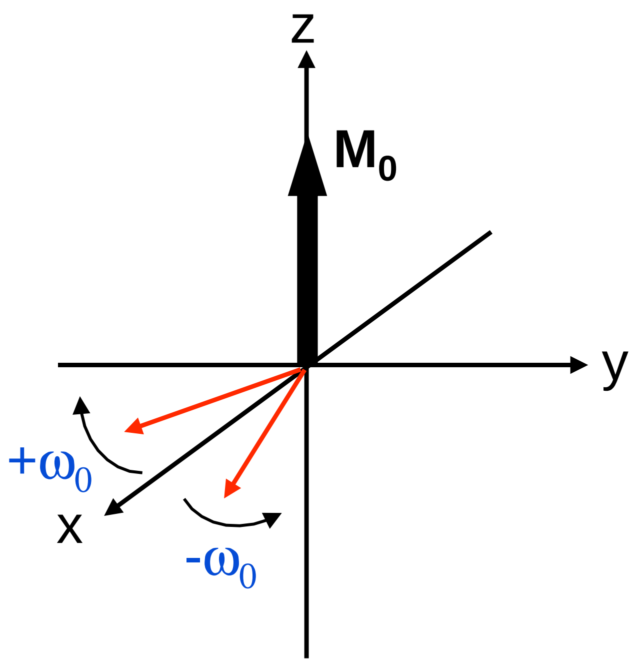




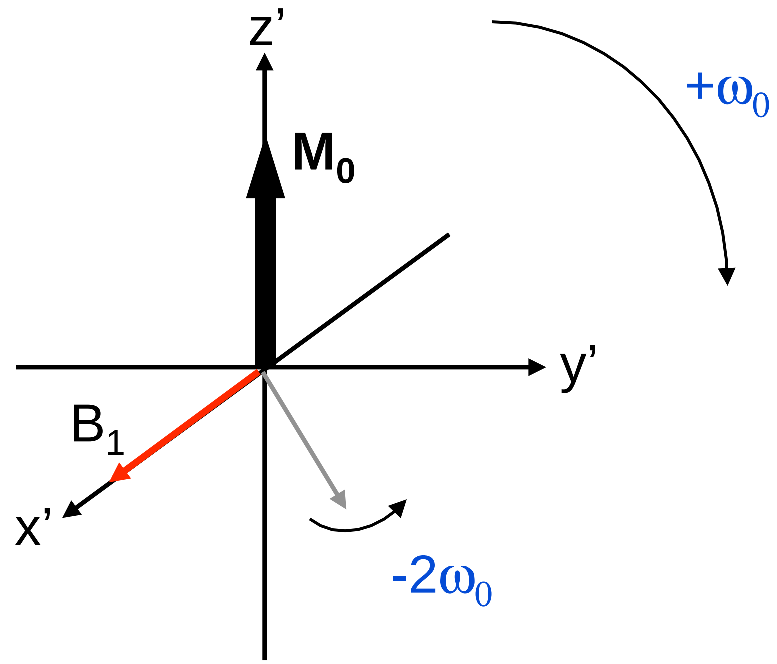
B_1 is an oscillating magnetic field

NMR Spectroscopy

Laboratory *vs.* Rotating frame



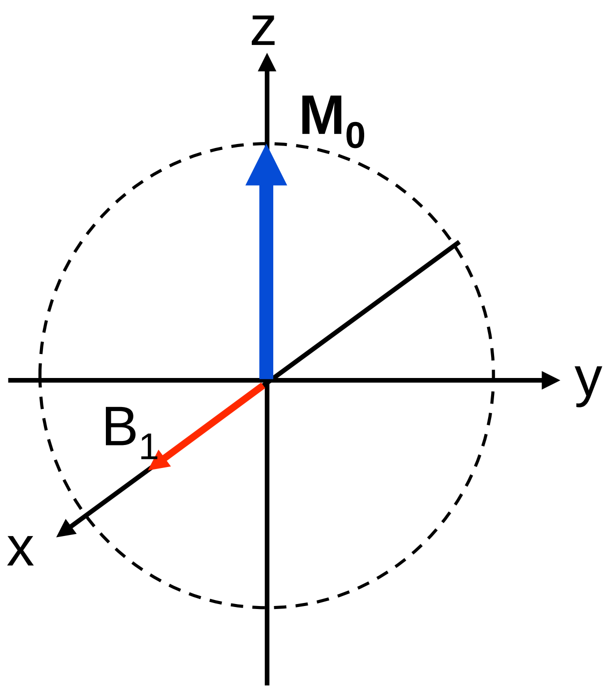
Laboratory frame

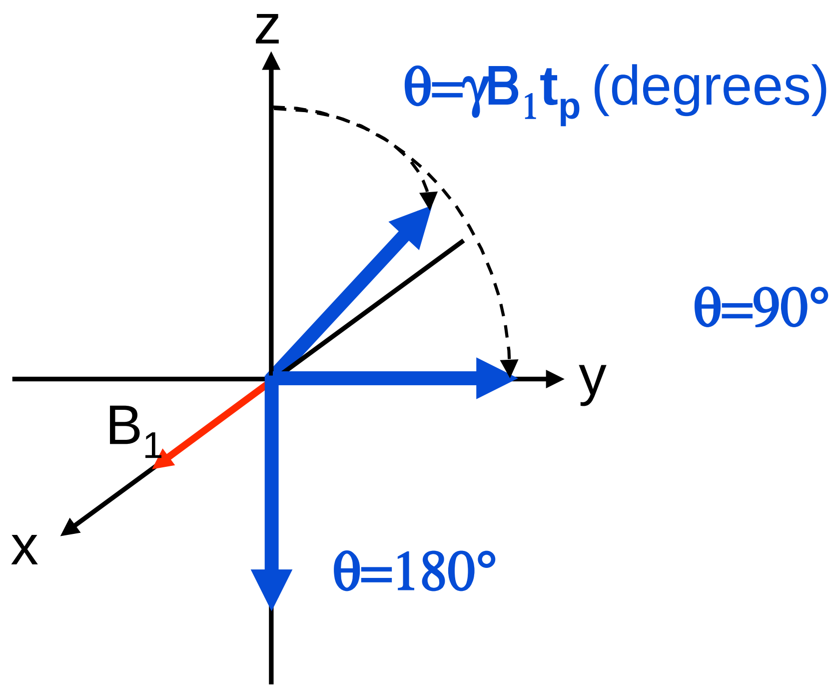


Rotating frame

NMR Spectroscopy

Effect on an ***rf*** pulse

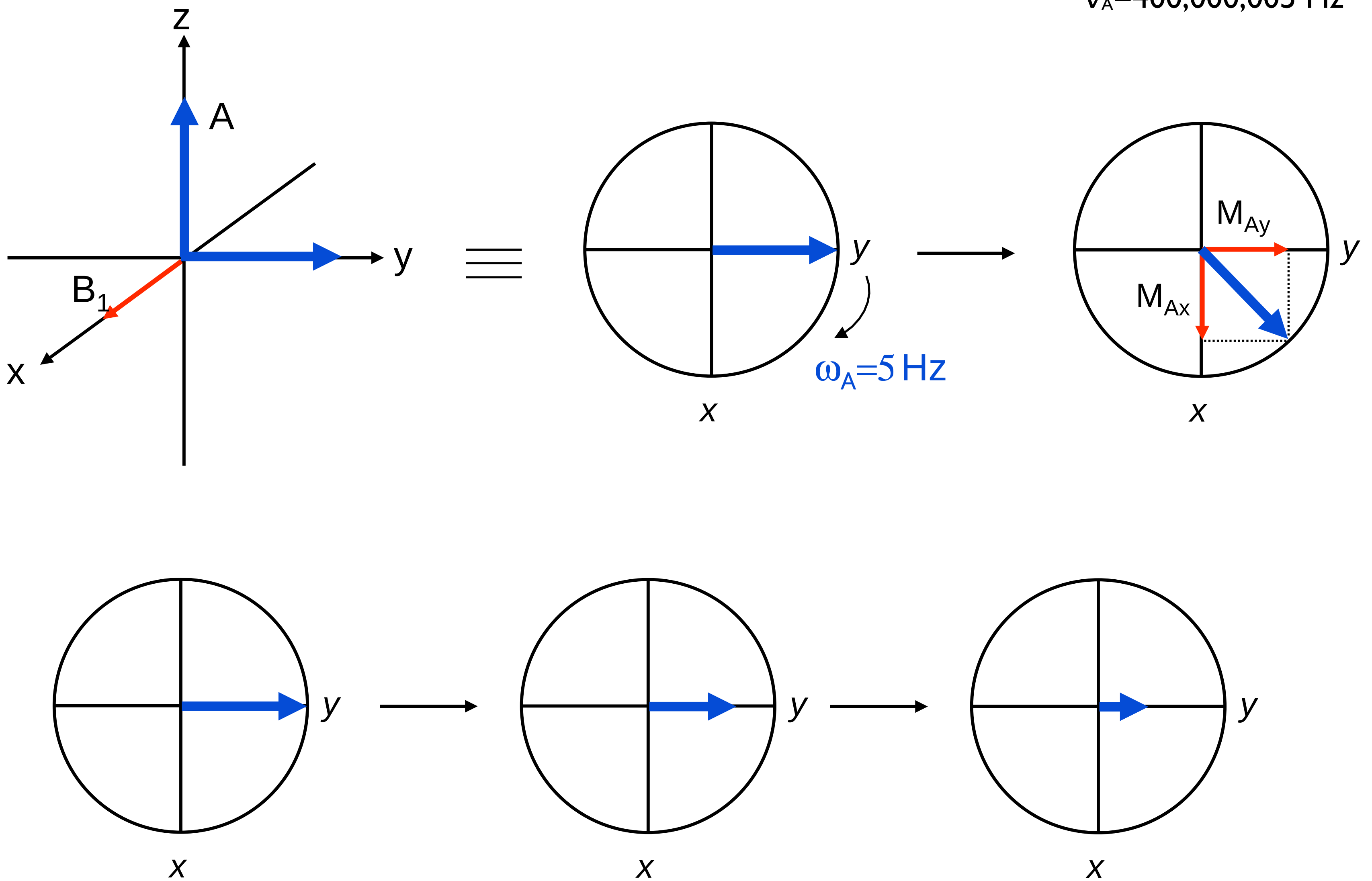




NMR Spectroscopy

Magnetization properties

$\nu_{^1H}$=400,000,000 Hz
ν_A=400,000,005 Hz



NMR Spectroscopy

Magnetization properties

ν_{1H}=400,000,000 Hz
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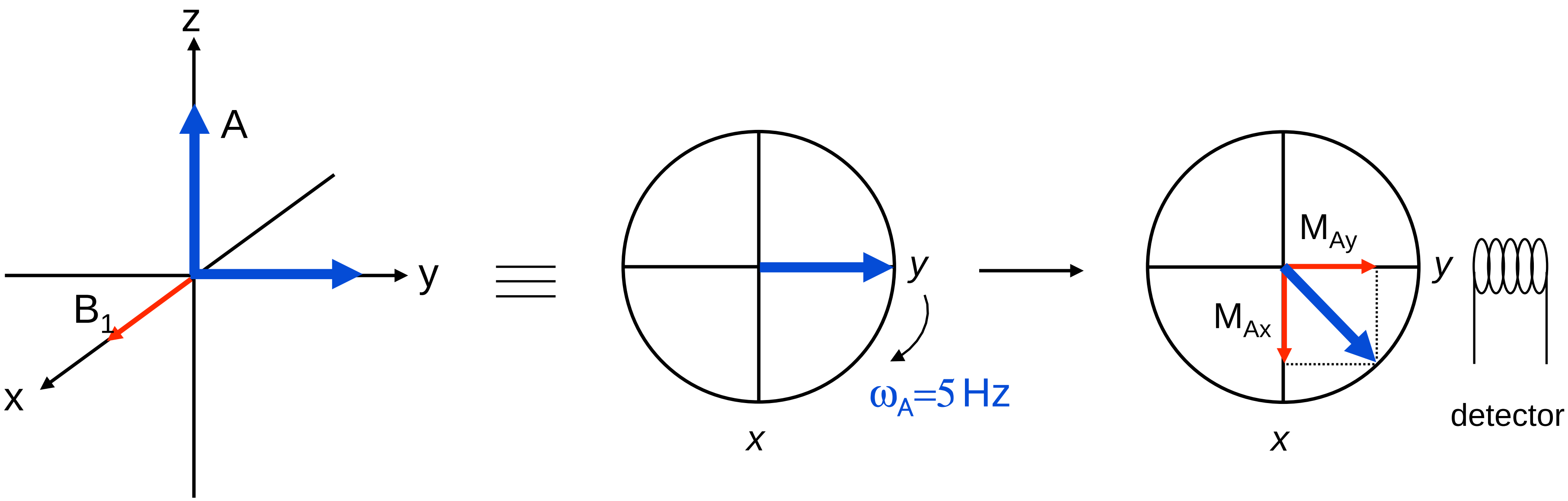
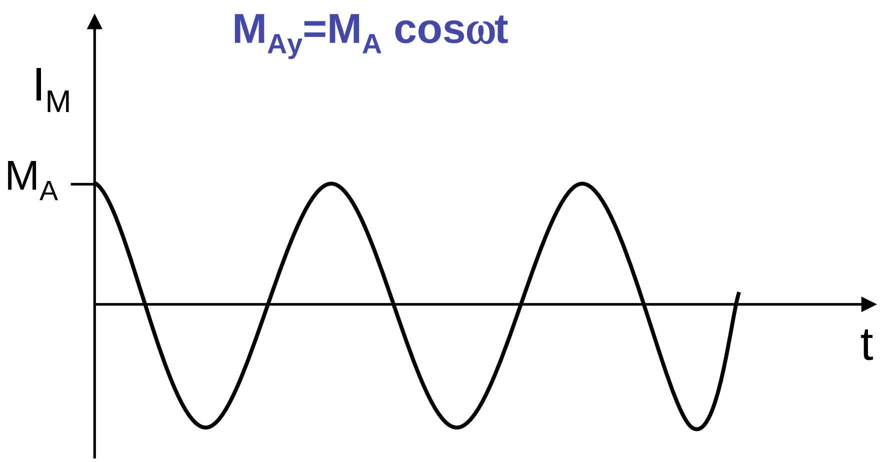
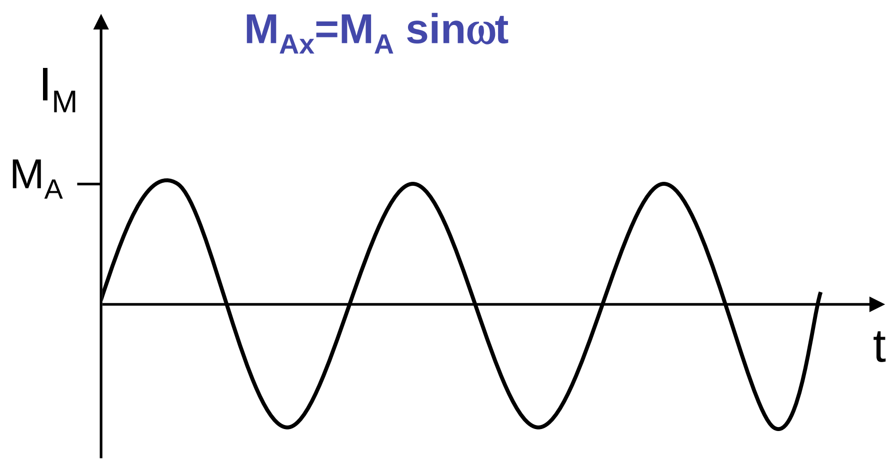


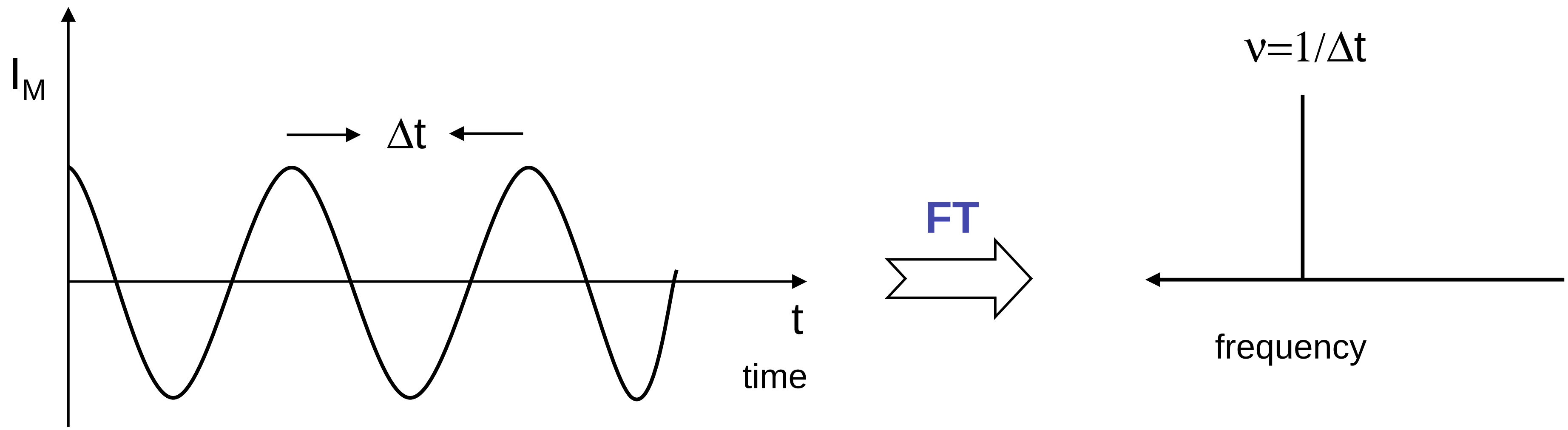




NMR Spectroscopy

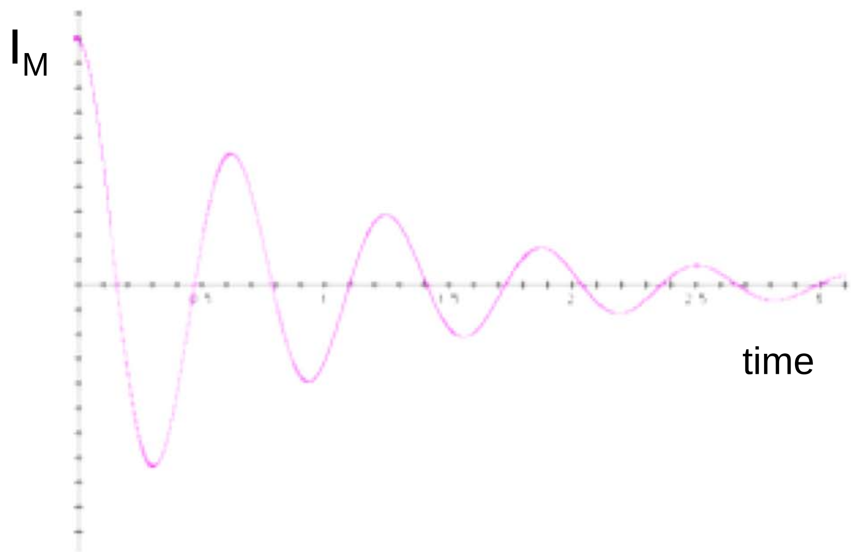
The Fourier Transform

time domain $\xrightarrow{\textbf{FT}}$ frequency domain

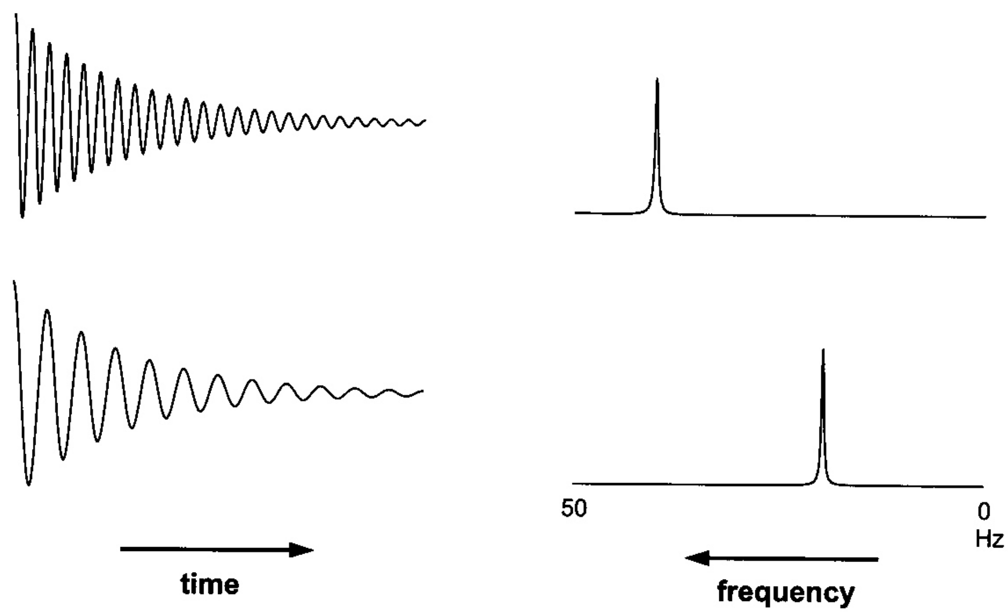


NMR Spectroscopy

The Fourier Transform

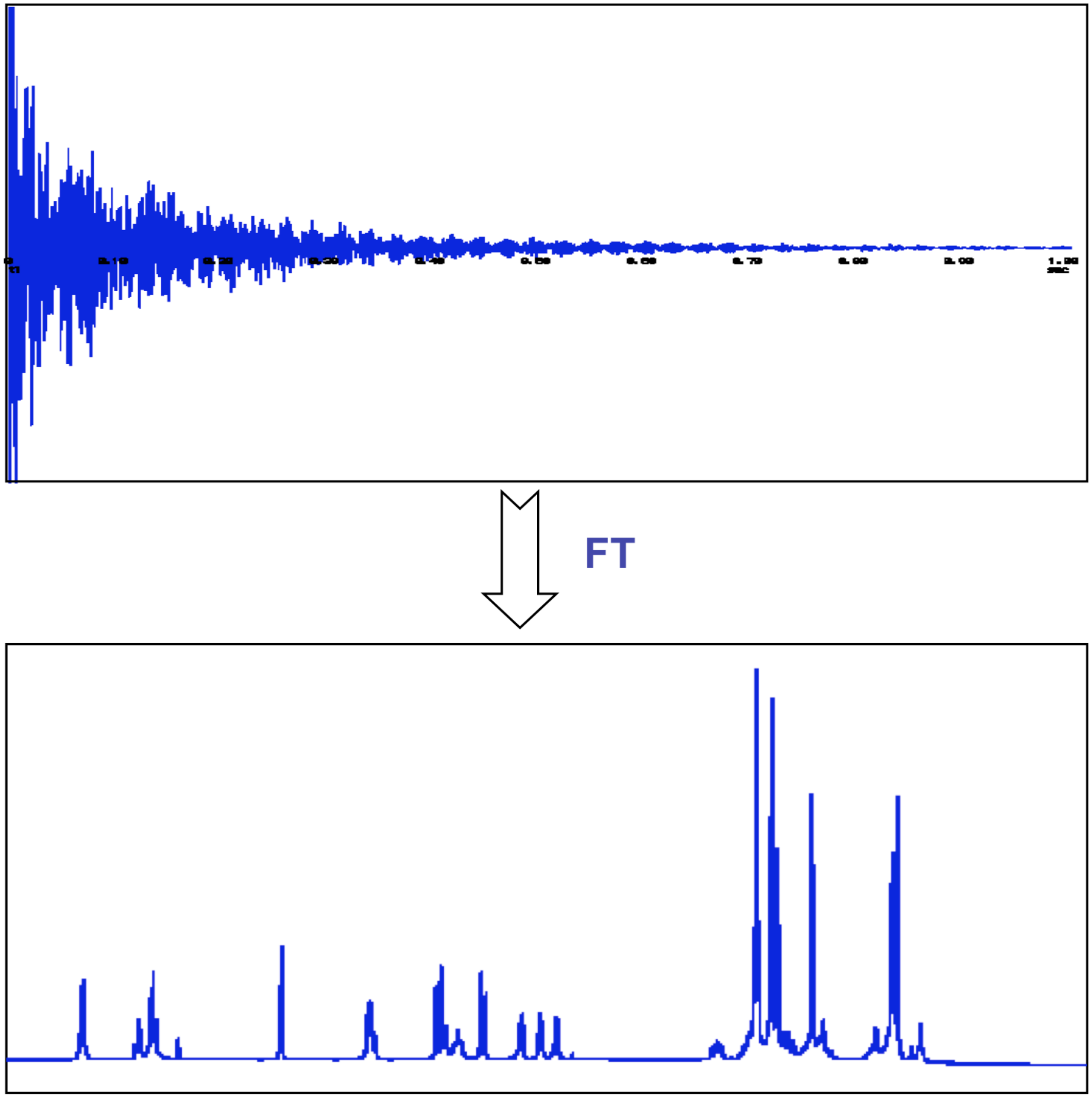


Signal Induction Decay (FID)



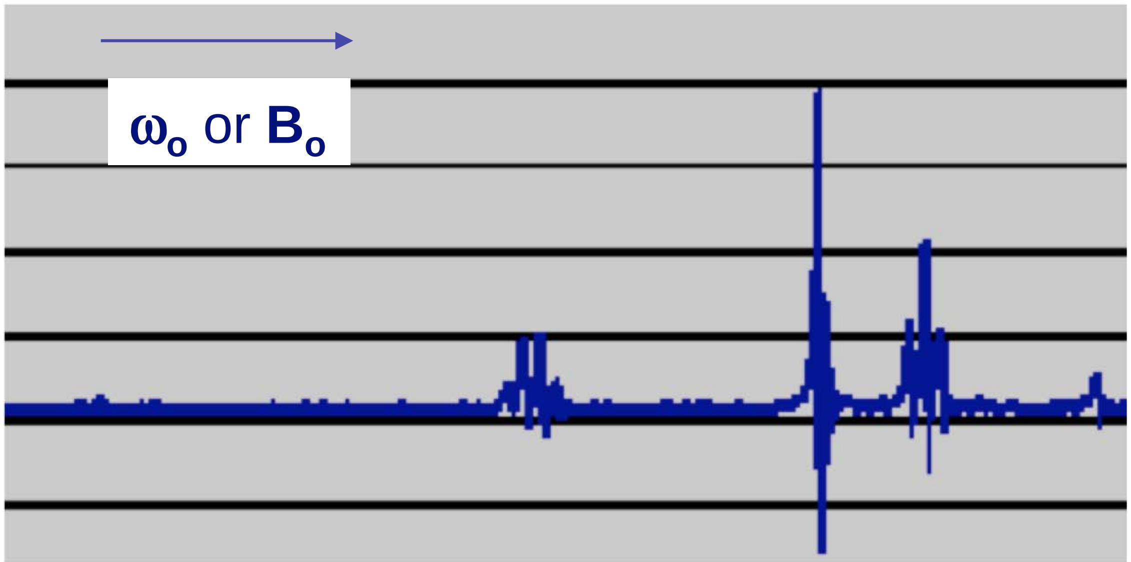
NMR Spectroscopy

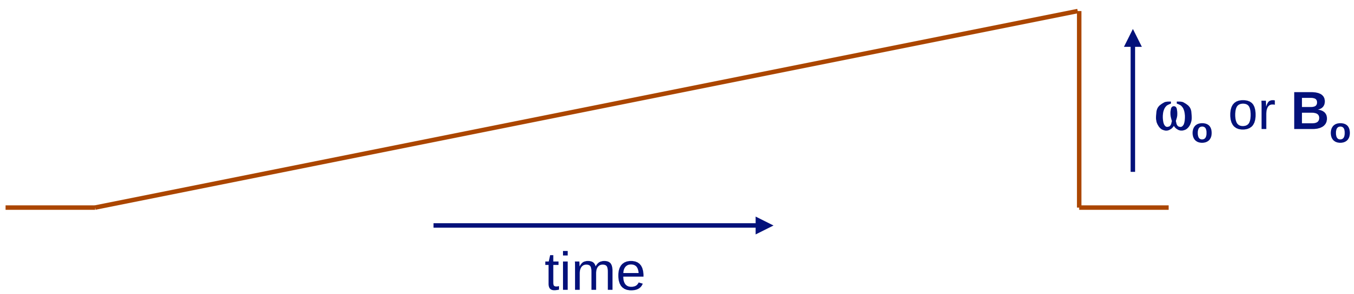
The Fourier Transform



NMR Spectroscopy

Continuous wave *vs.* pulsed NMR



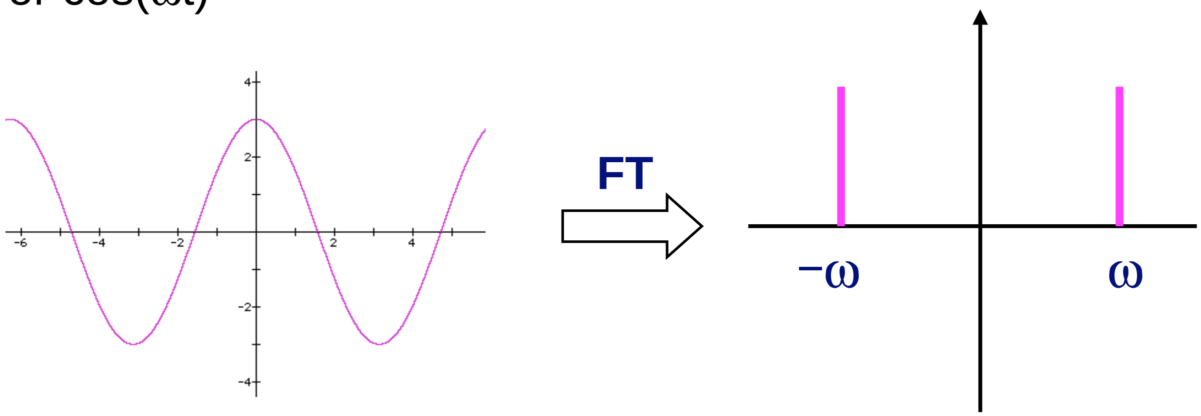


NMR Spectroscopy

Continuous wave *vs.* pulsed NMR

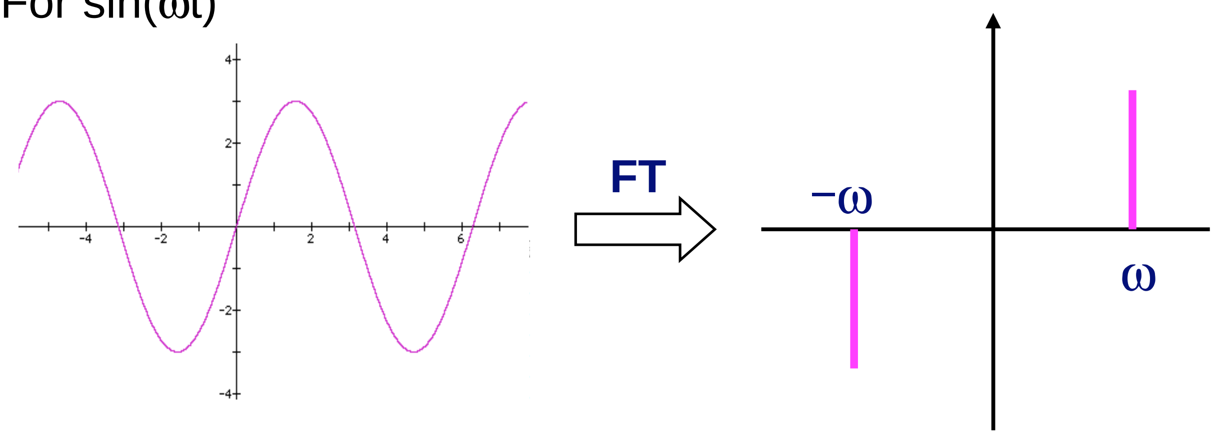
Fourier Transform of simple waves

• For cos(ωt)



absorptive lines

• For sin(ωt)

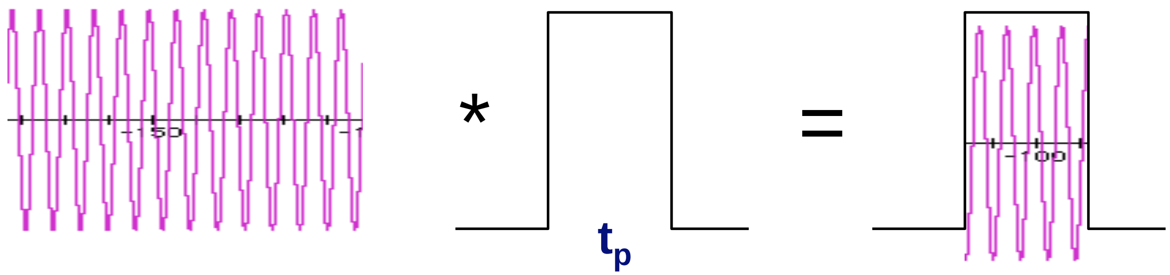


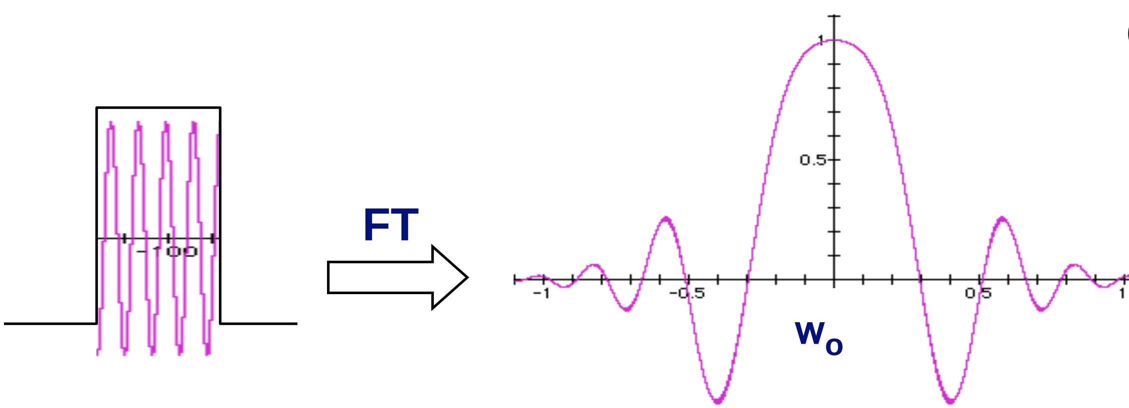
despersive lines

NMR Spectroscopy

Continuous wave *vs.* pulsed NMR

A **monochromatic** radiofrequency pulse is a combination of a wave (cosine) of frequency $\boldsymbol{\omega_0}$ and a step function



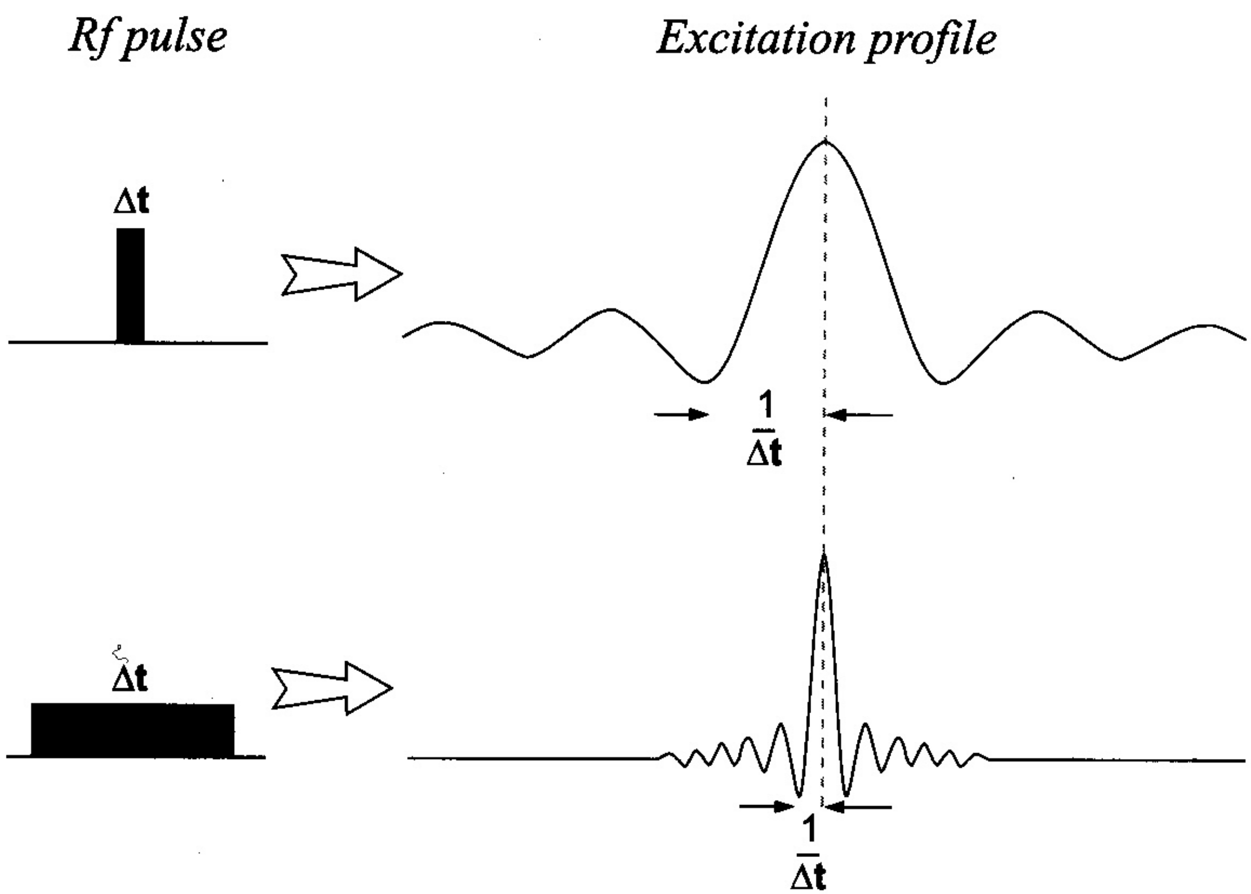


Since f=1/t, a pulse of **10 ms** duration excites a frequency bandwidth of **10^5 Hz**!

NMR Spectroscopy

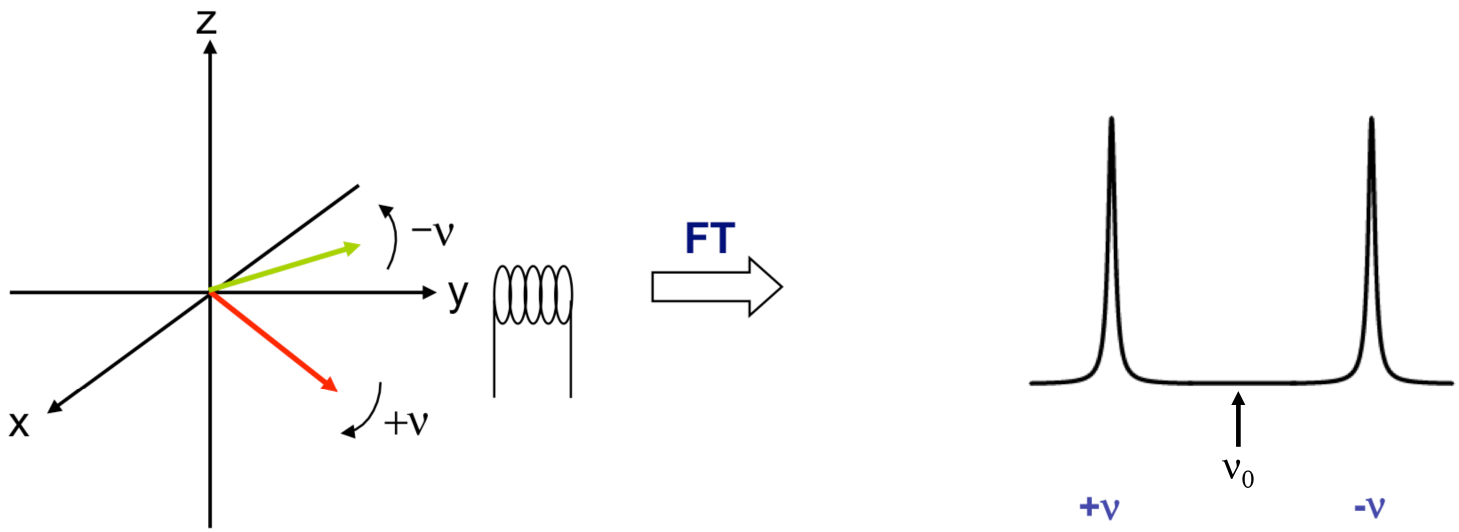
Continuous wave *vs.* pulsed NMR

$$\Delta E\ \Delta t \sim h \text{ or } \Delta \nu\ \Delta t \sim 1$$



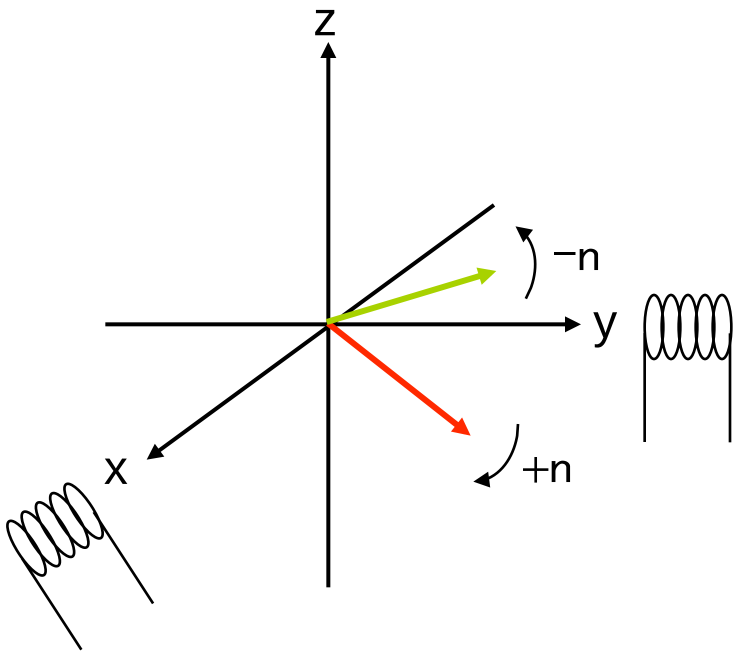
NMR Spectroscopy

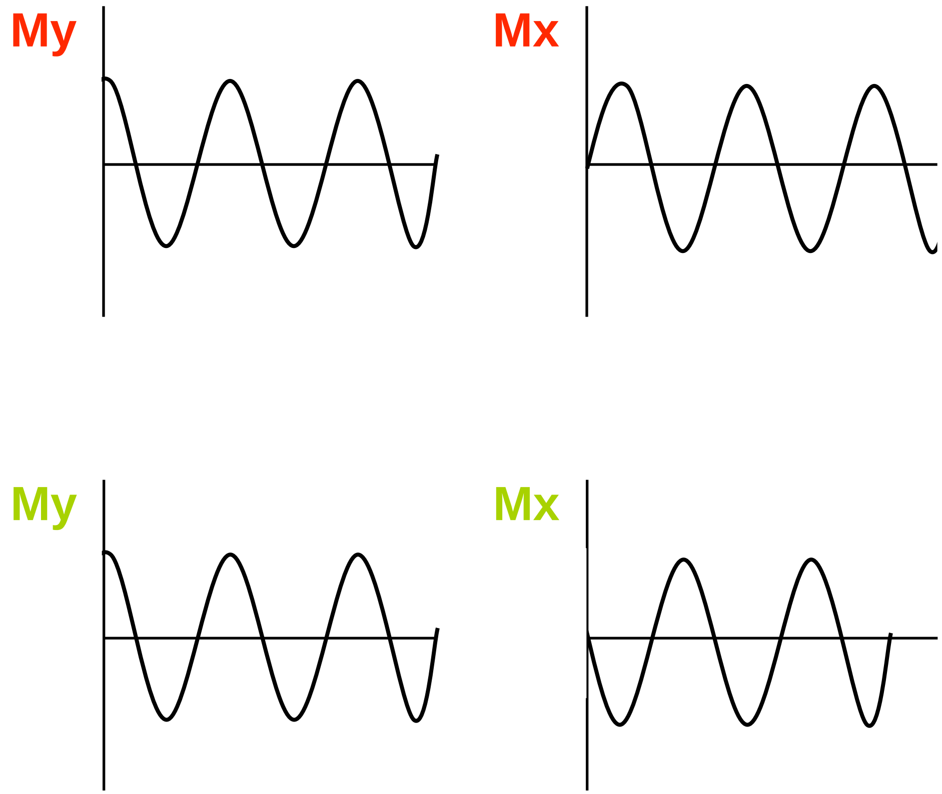
Single-channel signal detection



NMR Spectroscopy

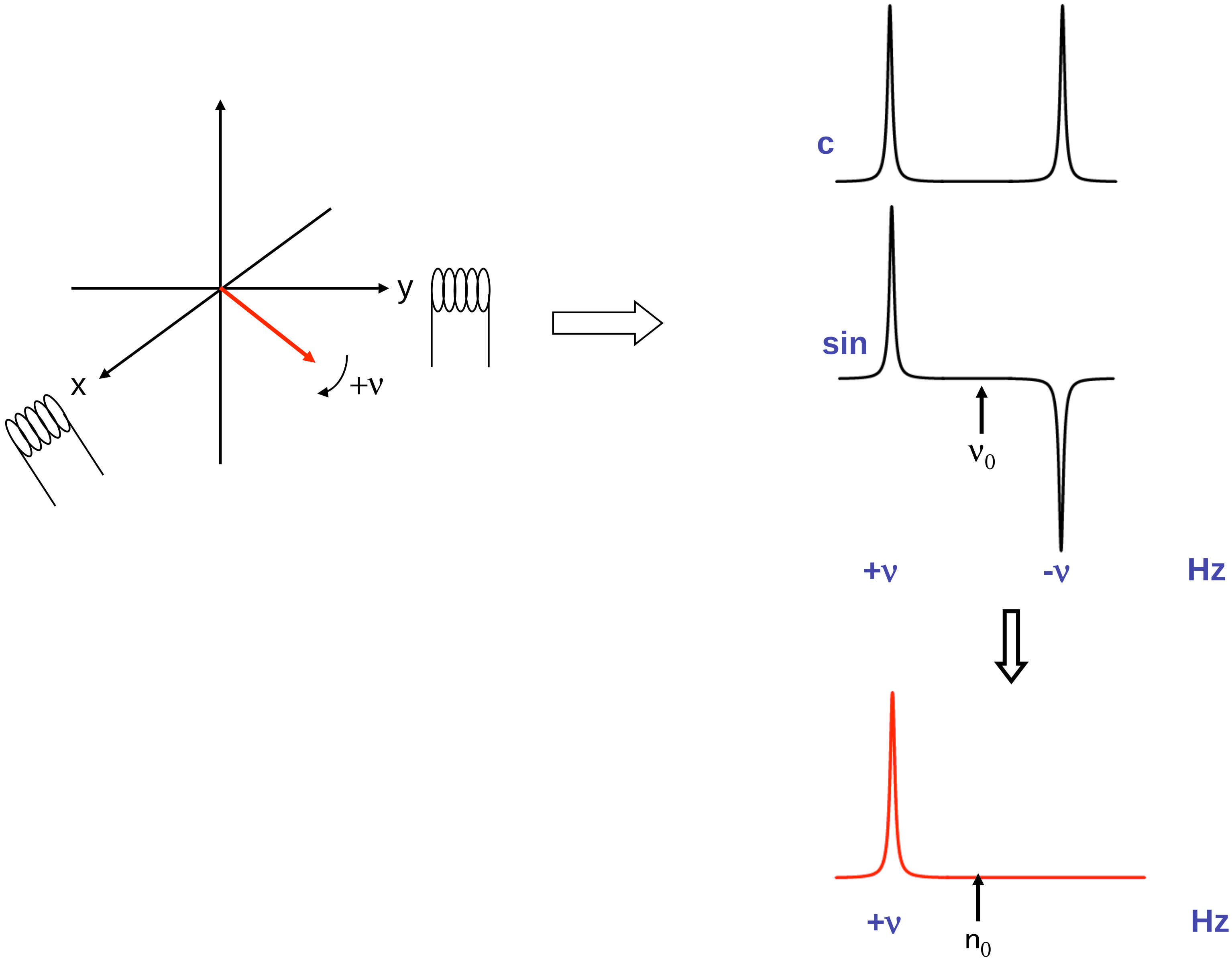
Quadrature detection





NMR Spectroscopy

Quadrature detection



NMR Spectroscopy

The Chemical Shift

The NMR frequency $\boldsymbol{\nu}$ of a nucleus in a molecule is mainly determined by its gyromagnetic ratio $\boldsymbol{\gamma}$ and the strength of the magnetic field **B**

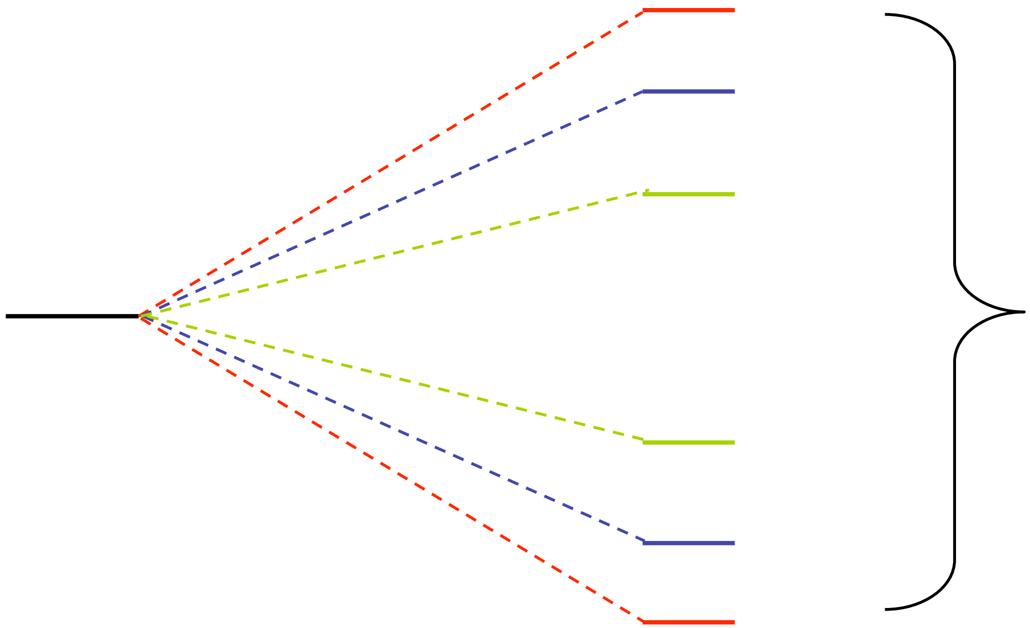
$$\nu = \frac{\gamma B}{2\pi}$$

The exact value of $\boldsymbol{\nu}$ depends, however, on the position of the nucleus in the molecule or more precisely on the local electron distribution

this effect is called the chemical shift

NMR Spectroscopy

The Chemical Shift



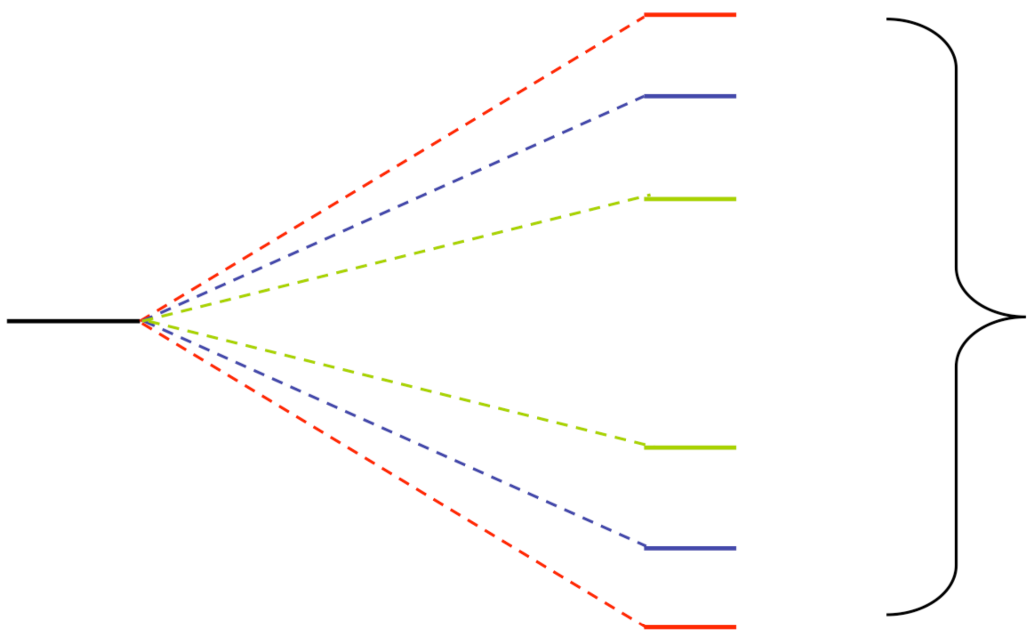
$\Delta E = h\nu = h\gamma B/2\pi$

Nuclei, however, in molecules are never isolated from other particles that are charged and are in motion (electrons!).

Thus, the field actually felt by a nucleus is slightly different from that of the applied external magnetic field!!

NMR Spectroscopy

The Chemical Shift



$\Delta E = h\nu = h\gamma B_{eff}/2\pi$

B_{eff}, is given by $B_0 - B' = B_0 - B_0\sigma = B_0(1-\sigma)$

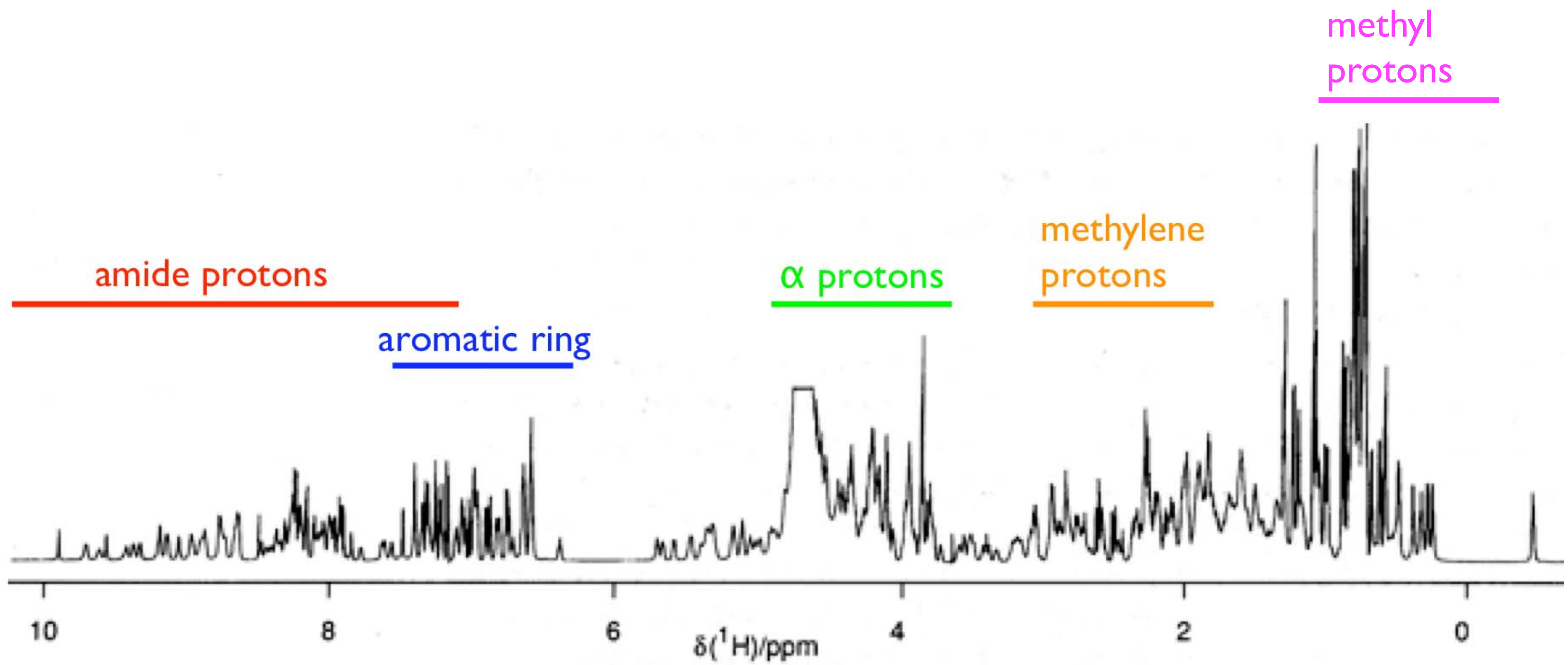
$$\nu = \frac{\gamma B_0(1-\sigma)}{2p}$$

and δ is the chemical shift

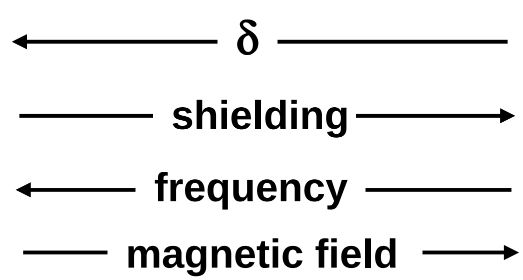
$$\delta = \frac{(\nu - \nu_{ref})}{\nu_{ref}} 10^6 \approx 10^6 (\sigma_{ref} - \sigma)$$

NMR Spectroscopy

The Chemical Shift

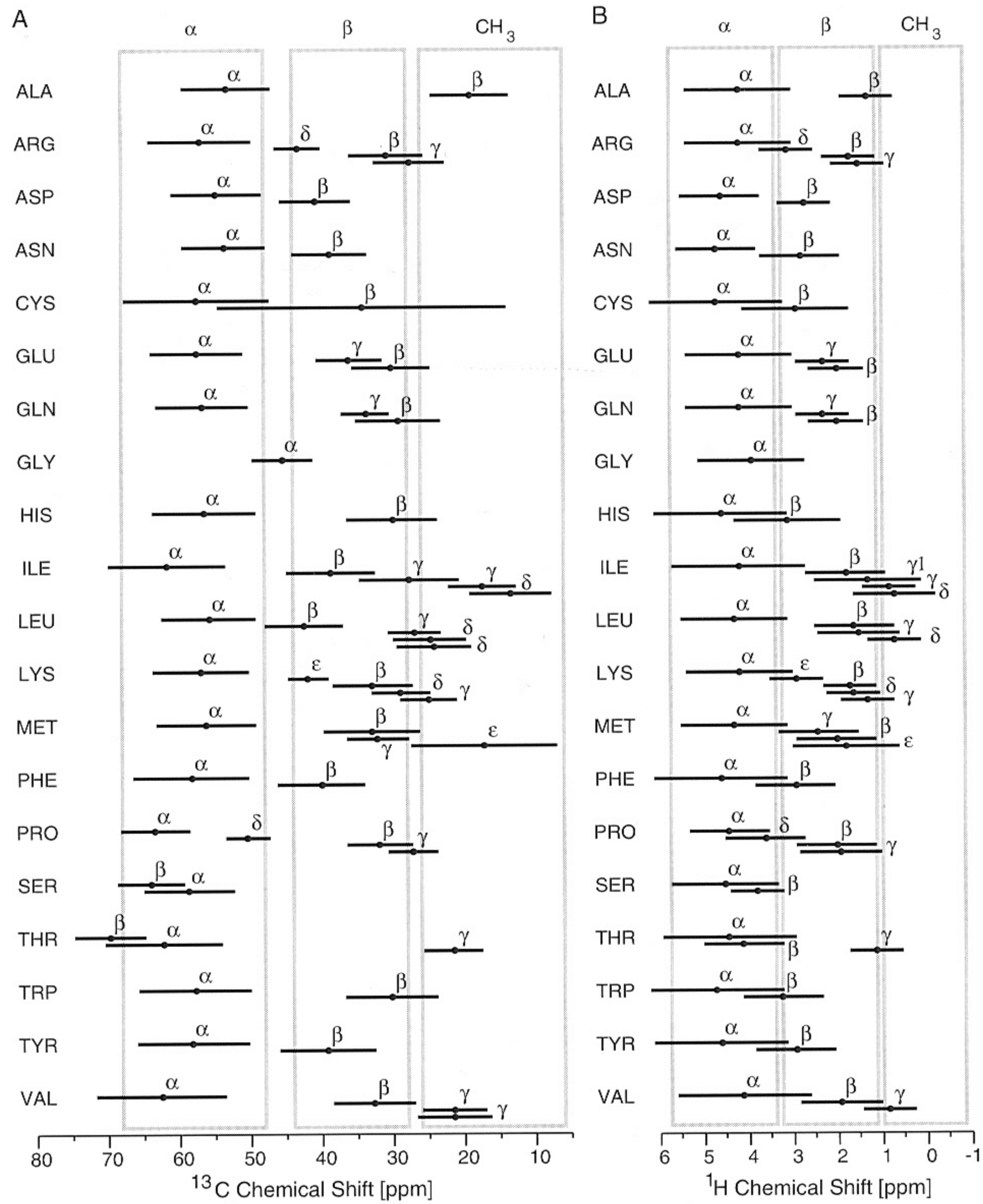


750 MHz ^{1}H spectrum of a small protein



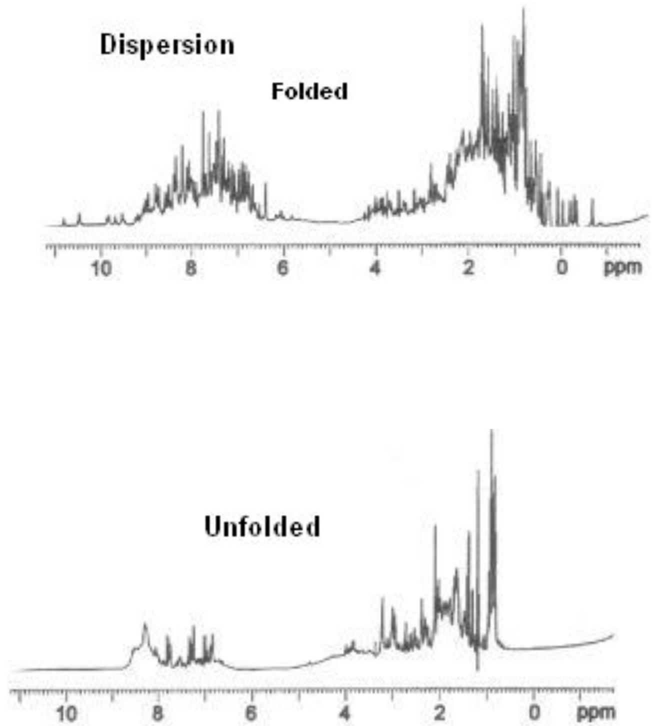
NMR Spectroscopy

The Chemical Shift



NMR Spectroscopy

The Chemical Shift



NMR Spectroscopy

Nuclear Shielding

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

diamagnetic contribution

paramagnetic contribution

neighbor anisotropy effect

ring-current effect

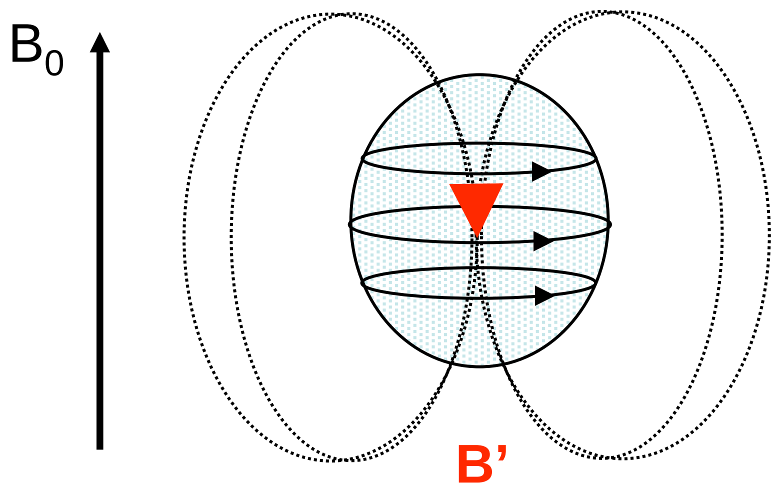
electric field effect

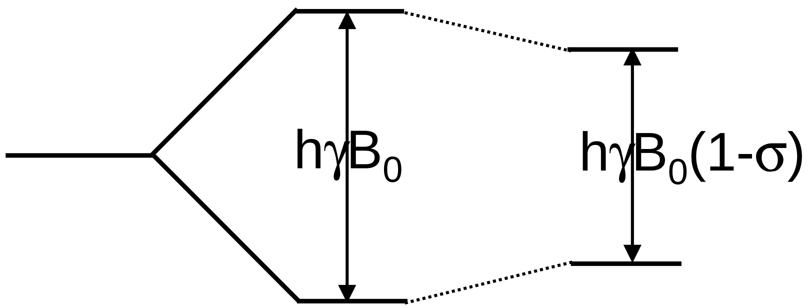
solvent effect

NMR Spectroscopy

Nuclear Shielding - diamagnetic contribution

The external field B_0 causes the electrons to circulate within their orbitals





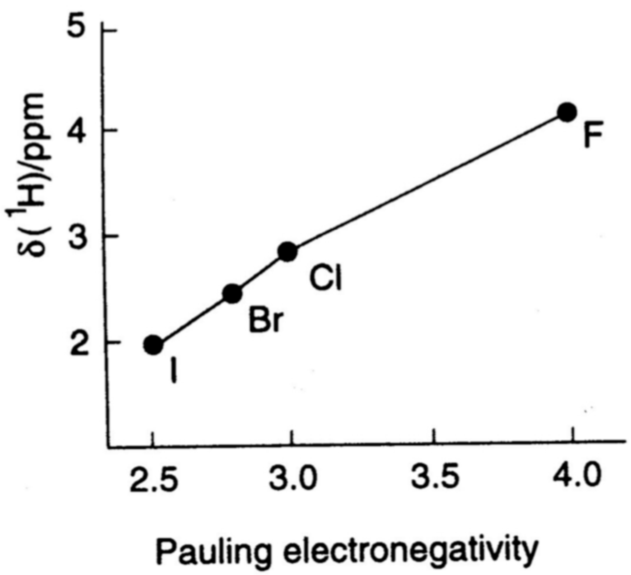
The higher is the electron density close to the nucleus, the larger the protection is!

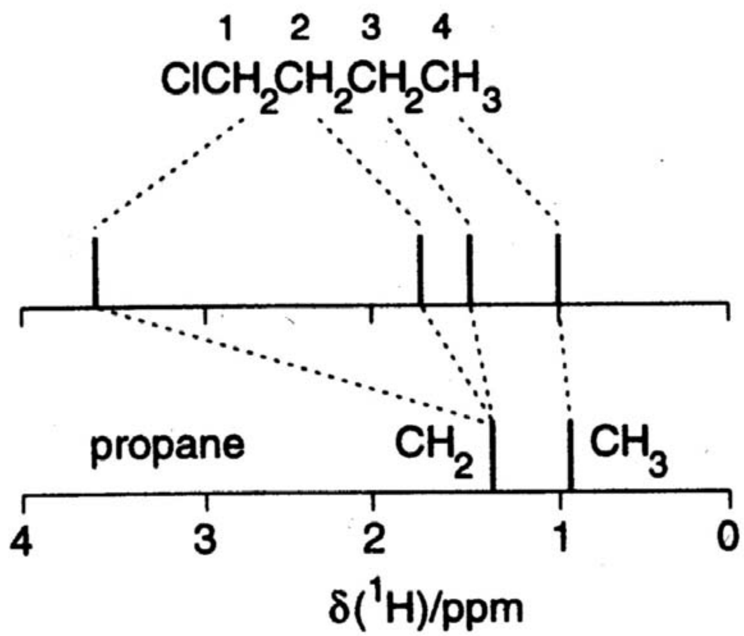
NMR Spectroscopy

Nuclear Shielding - diamagnetic contribution

Depends on the electronegativity

CH_3X



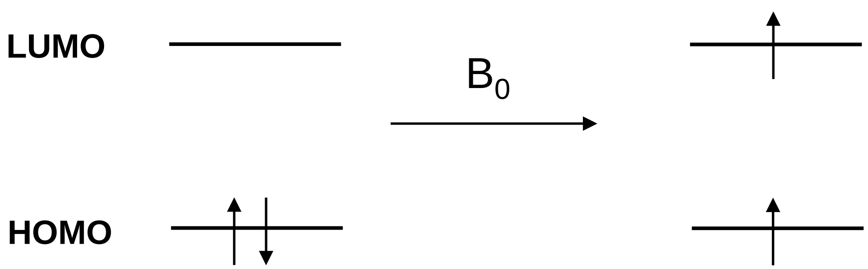


NMR Spectroscopy

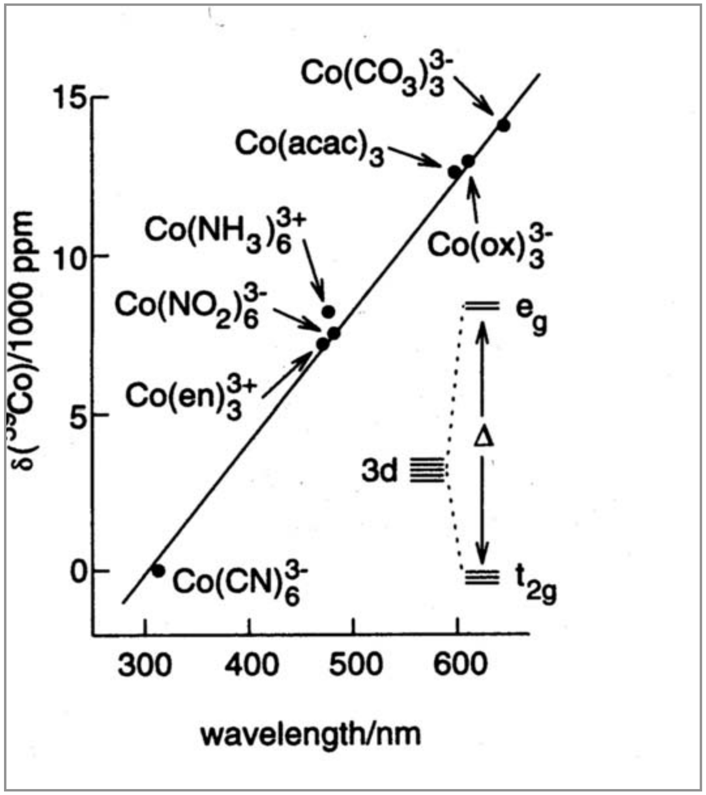
Nuclear Shielding - paramagnetic contribution

The external field B_0 mixes the wavefunction of the ground state with that of the excited state

The induced current generates a magnetic field that enhances the external field and deshields the nucleus



$$\sigma_p = \frac{1}{\Delta E}\left\langle \frac{1}{R^3} \right\rangle$$



NMR Spectroscopy

Chemical shift range

^{1}H; ~10 ppm

^{13}C; ~200 ppm

^{19}F; ~300 ppm

^{31}P; ~500 ppm

Local diamagnetic and paramagnetic currents make only modest contributions to ^{1}H shielding!

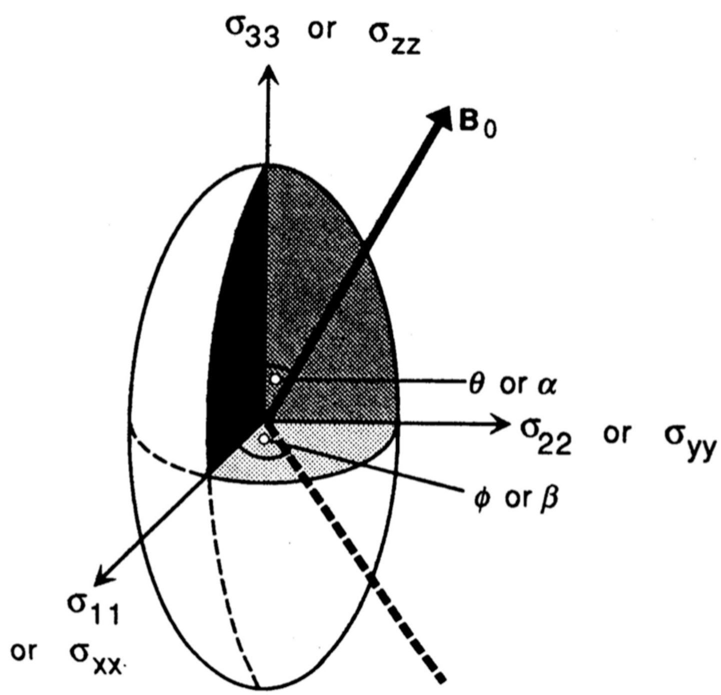
NMR Spectroscopy

Chemical Shift Anisotropy

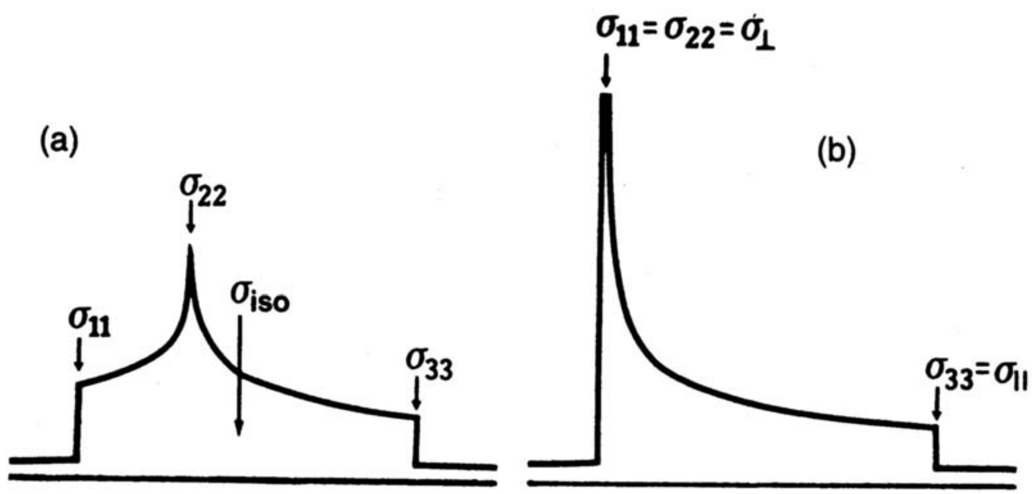
Nuclear shielding, σ, is a tensor.

The distribution of the electrons about the nucleus is non-sperical- thus, the magnitude of the shielding depends on the relative orientation of the nucleus with respect to the static field.

In isotropic cases: $\sigma = \frac{1}{3}(\sigma_{11} + \sigma_{22} + \sigma_{33})$

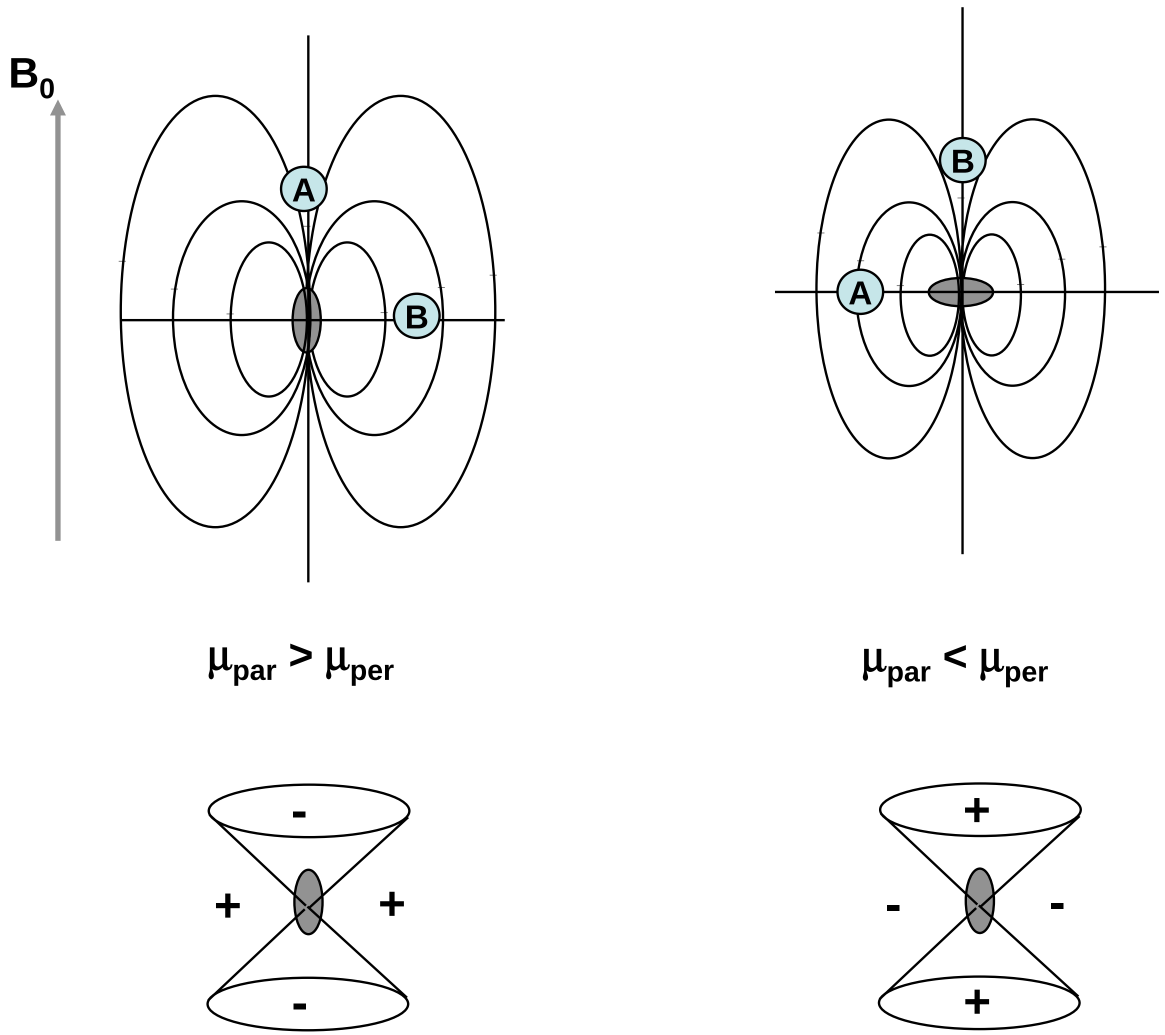


In static cases, e.g. solid state



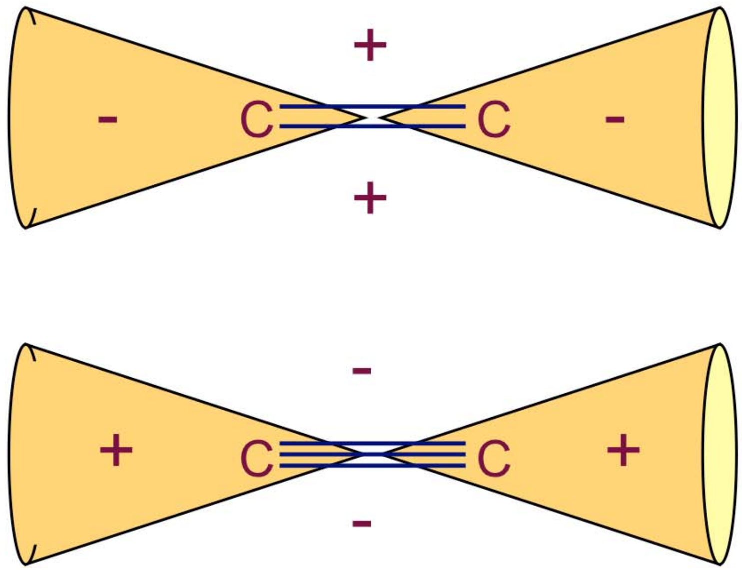
NMR Spectroscopy

Nuclear Shielding - neighboring group



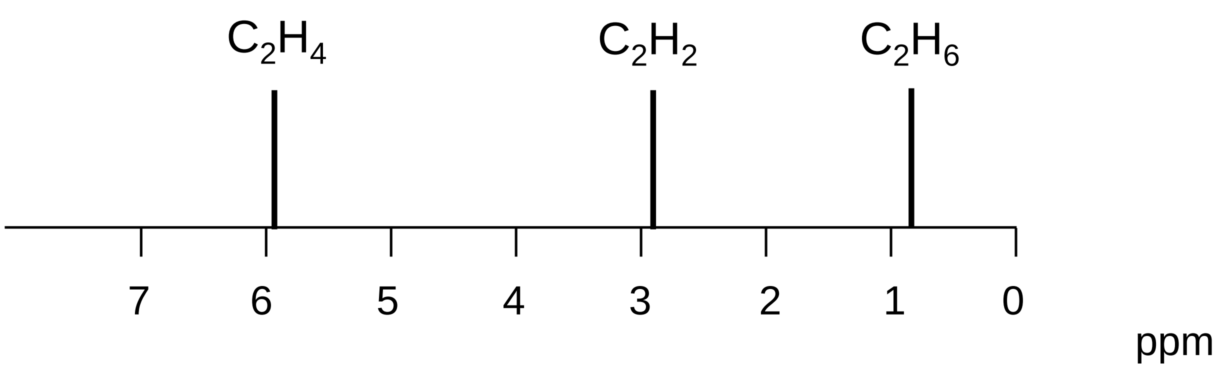
NMR Spectroscopy

Nuclear Shielding - neighboring group



$\mu_{par} > \mu_{per}$

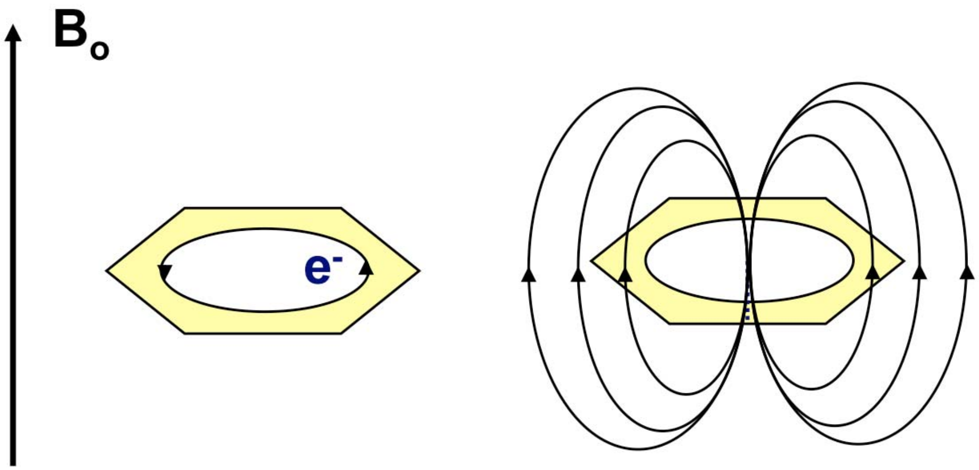
$\mu_{par} < \mu_{per}$

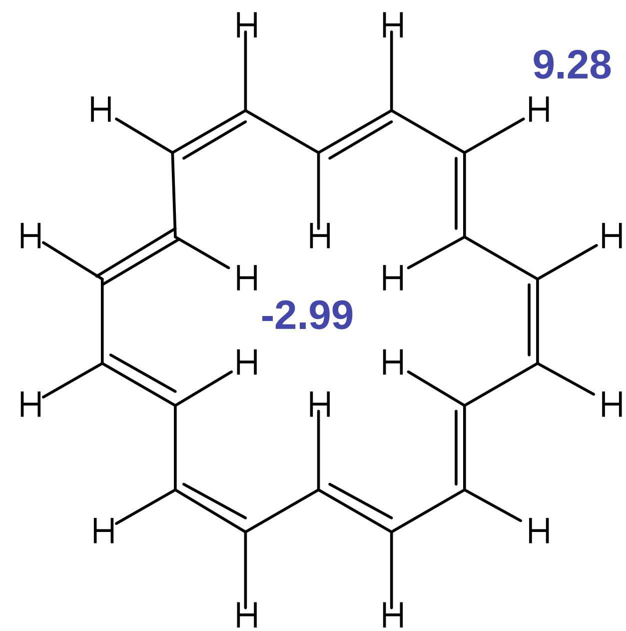


NMR Spectroscopy

Nuclear Shielding - ring-current effect

More pronounced in aromatic rings due to the π electron clouds

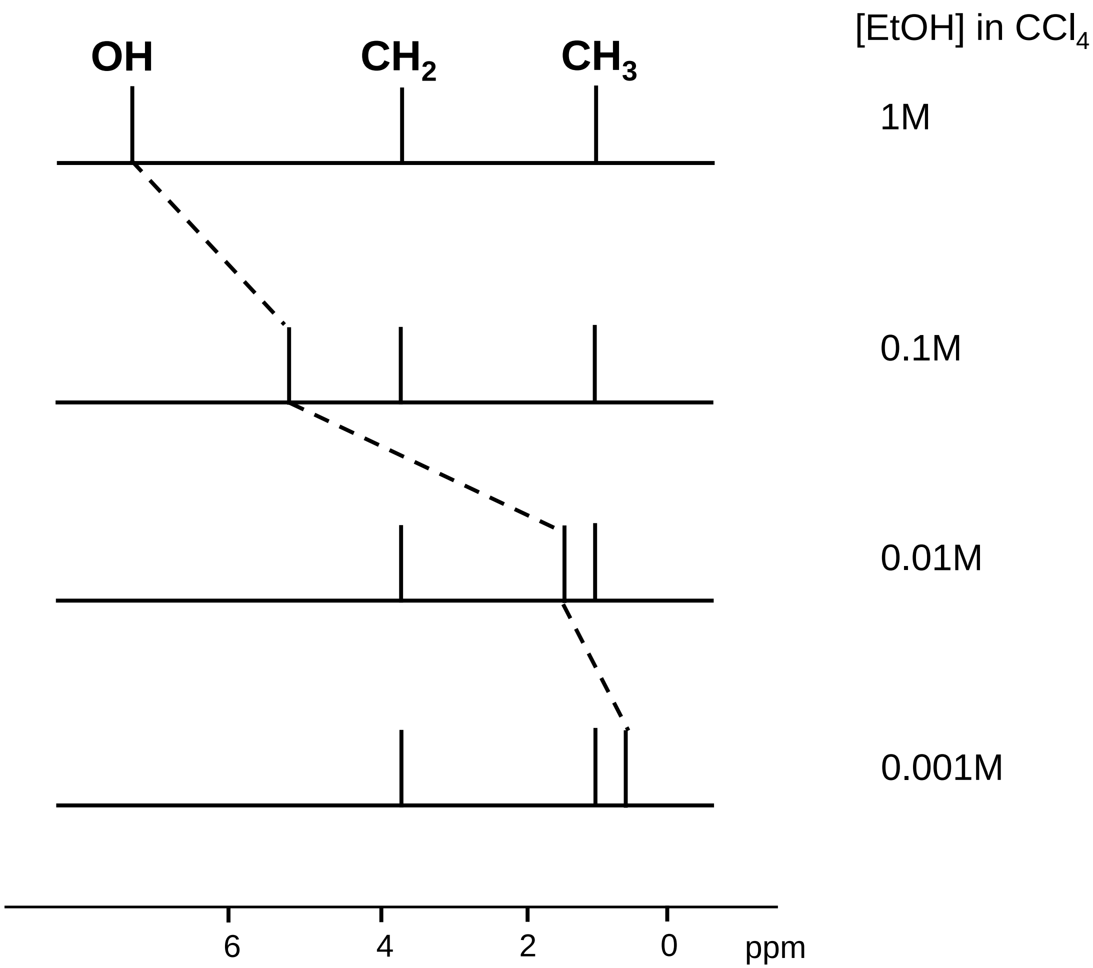




NMR Spectroscopy

Nuclear Shielding - hydrogen bonding

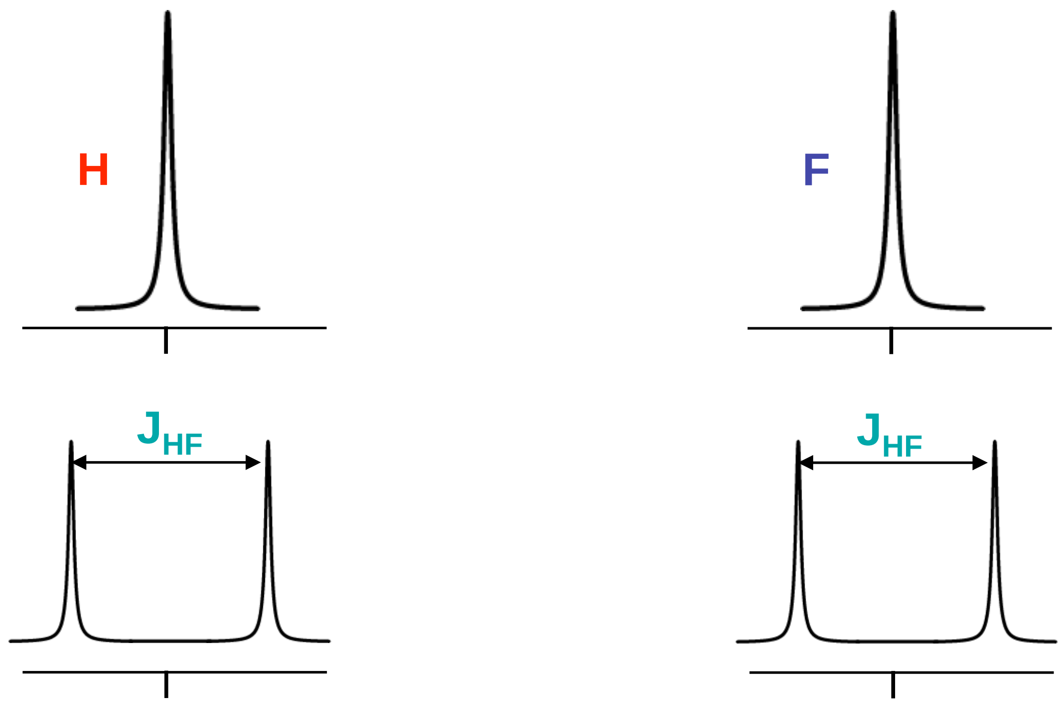
Hydrogen bonding causes deshielding due to electron density decrease at the proton site



NMR Spectroscopy

Spin-spin (scalar) coupling

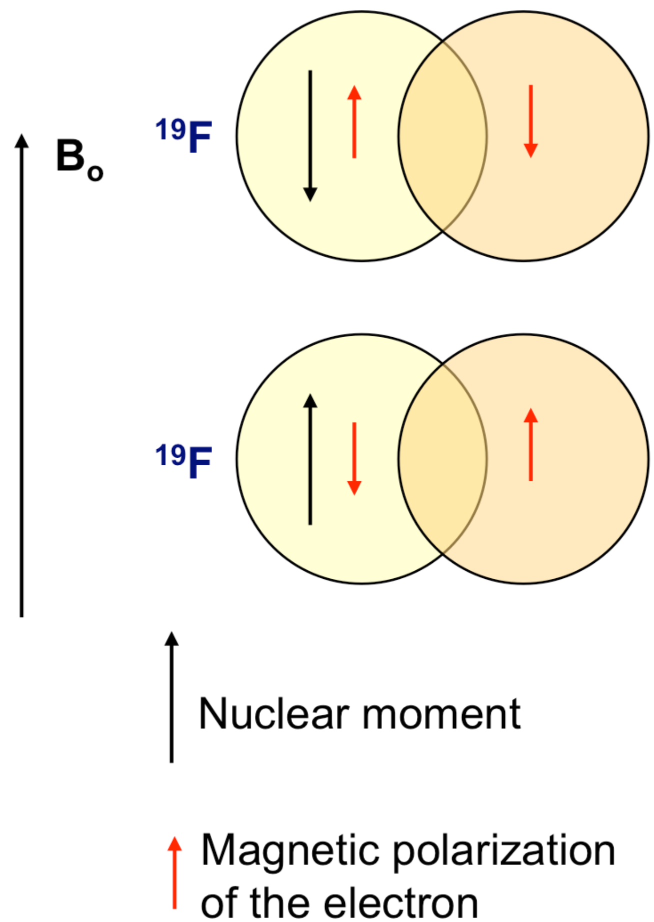
HF (^{1}H-^{19}F)

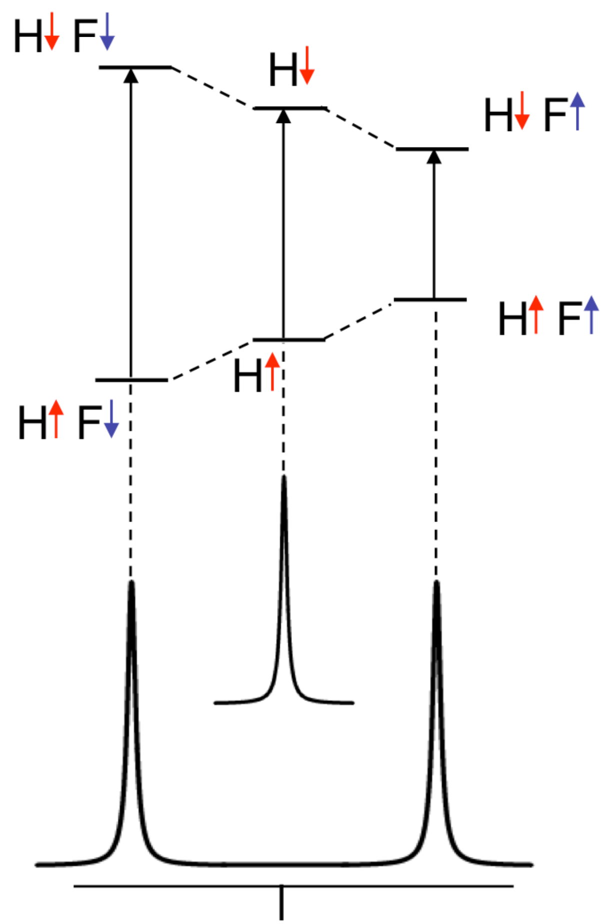


NMR Spectroscopy

Spin-spin (scalar) coupling

HF (^{1}H-^{19}F)





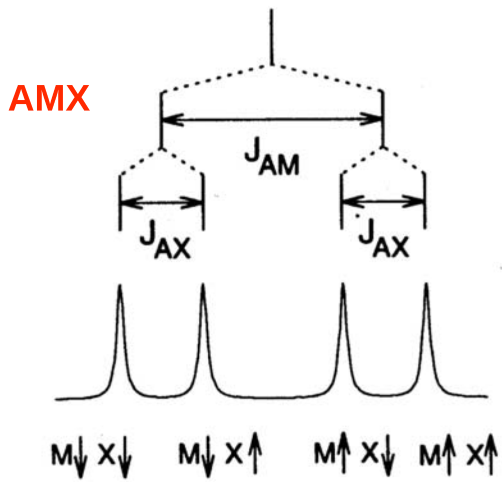
$$E = h\, J_{AX}\, m_A\, m_X$$

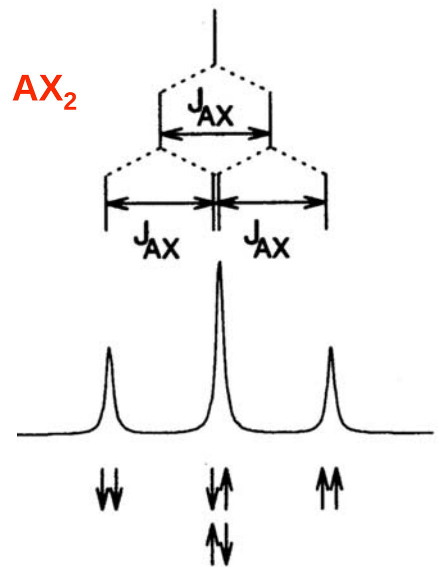
where **m** is the magnetic quantum number

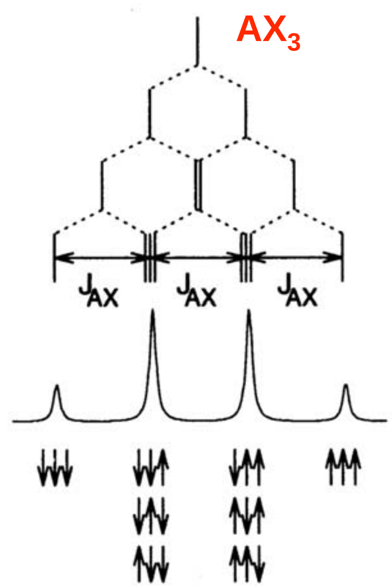
J_{AX} is the spin-spin coupling constant

NMR Spectroscopy

Spin-spin (scalar) coupling

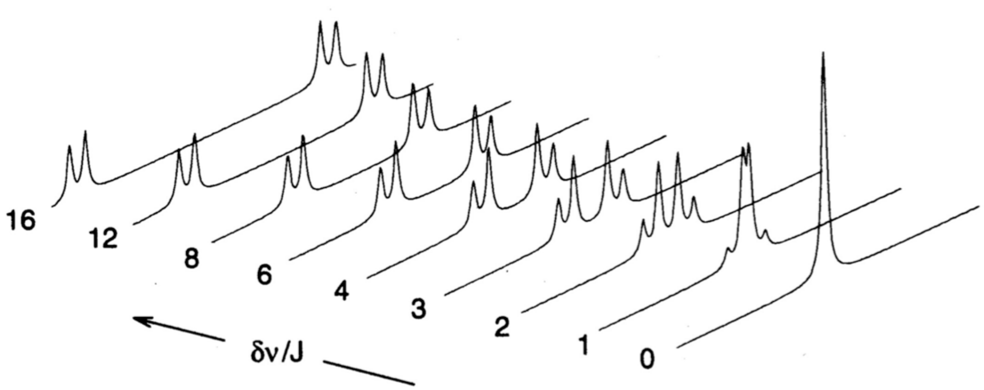


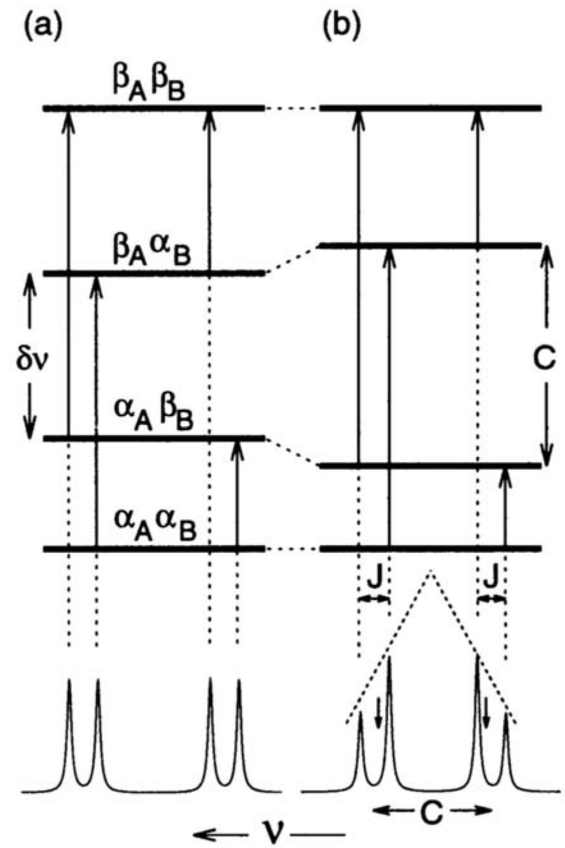




NMR Spectroscopy

Spin-spin (scalar) coupling





Strong coupling – **$\delta\nu<10|J|$**

NMR Spectroscopy

Spin-spin (scalar) coupling

The principal source of scalar coupling is an indirect interaction mediated by **electrons** involved in chemical bonding

The **magnitude** of interaction is proportional to the **probability** of finding the electron at the nucleus (R=0)

Magnitude in **Hz**- **independent** of the external magnetic field

$H_3C - CH_3$	125 Hz
$H_2C = CH_2$	160 Hz
$HC \equiv CH$	250 Hz

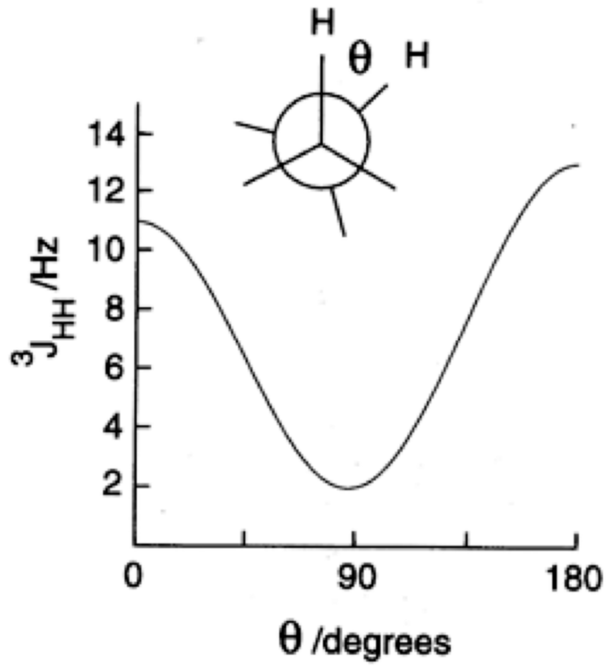
NMR Spectroscopy

Spin-spin (scalar) coupling

Three-bond coupling most useful since it carries information on dihedral angles

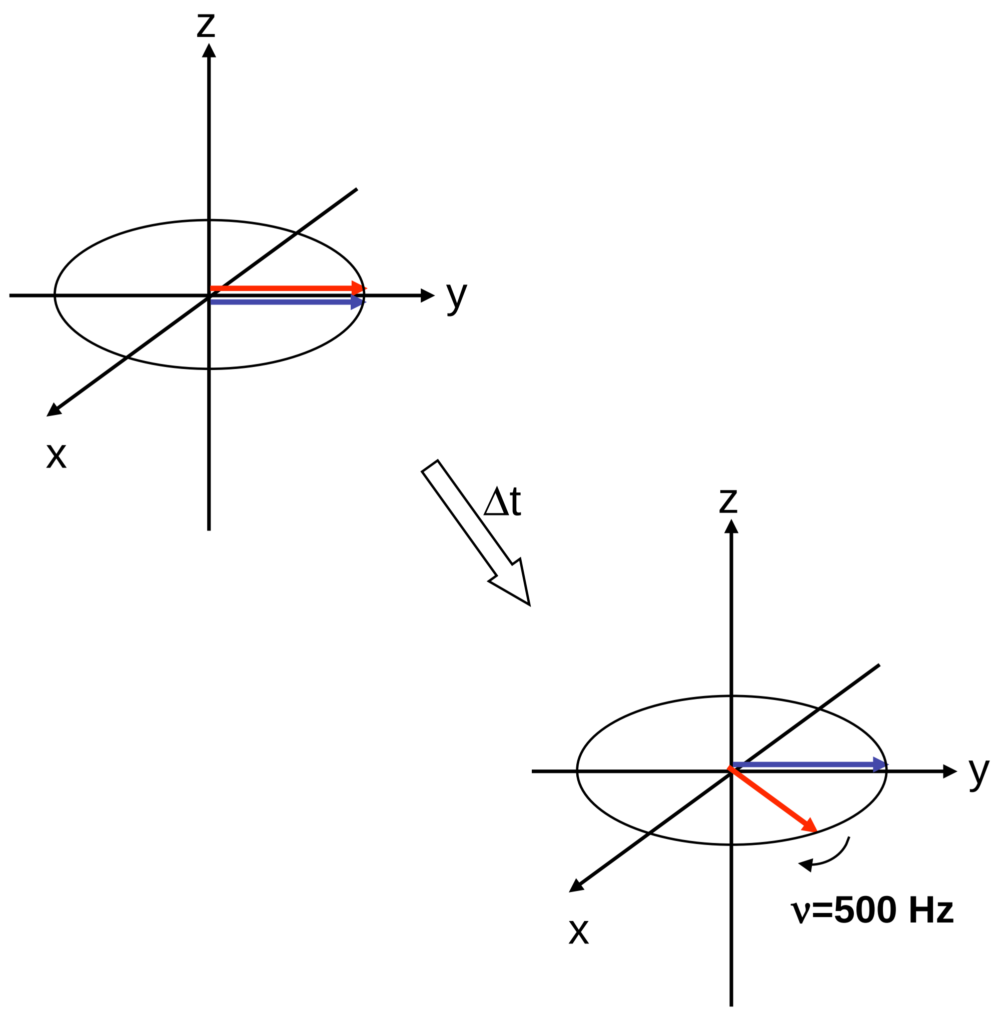
Empirical relationship: the Karplus relation

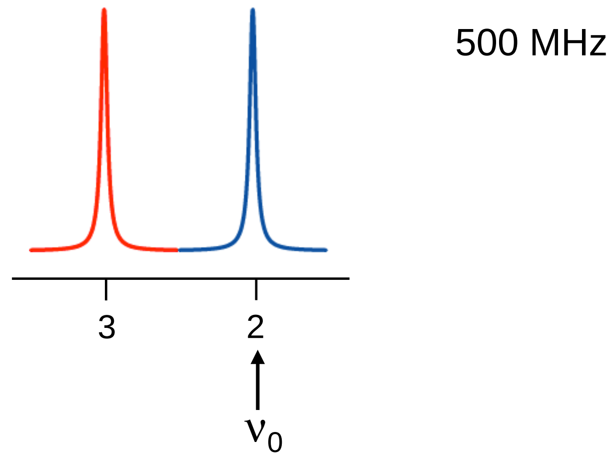
$$^{3}J = A + B\cos\theta + C\cos^{2}\theta$$



NMR Spectroscopy

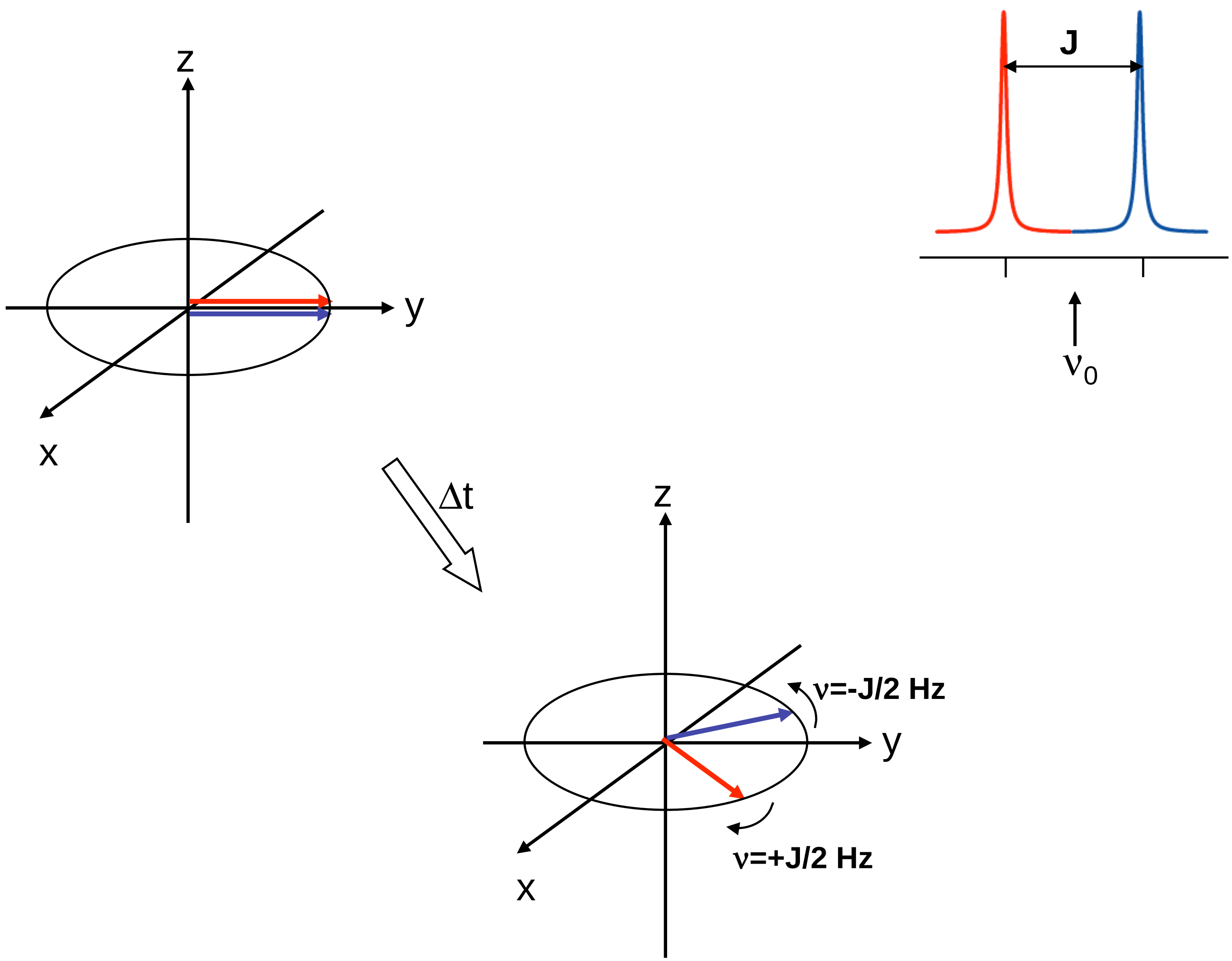
Chemical shifts on the rotating frame





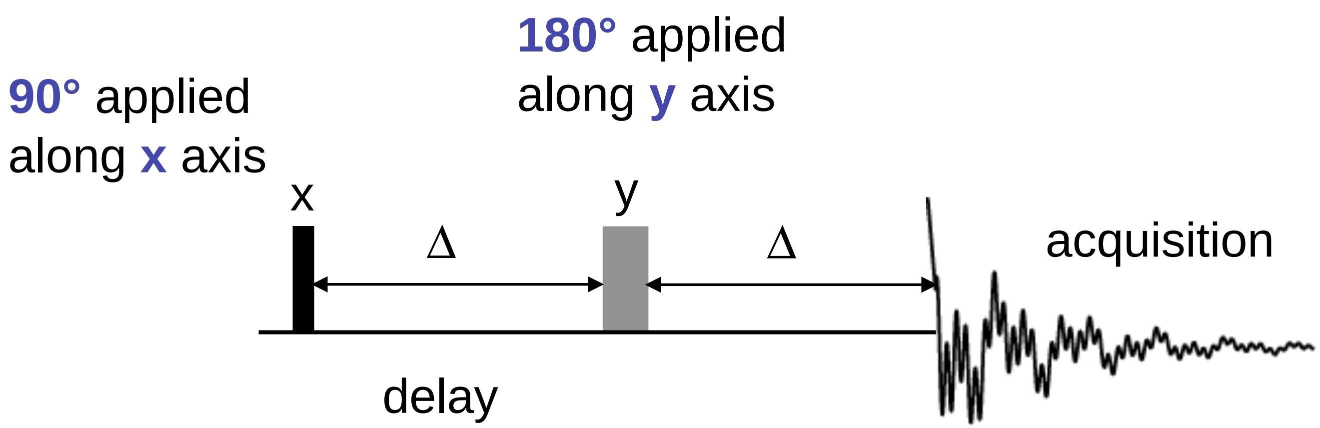
NMR Spectroscopy

Spin couplings on the rotating frame



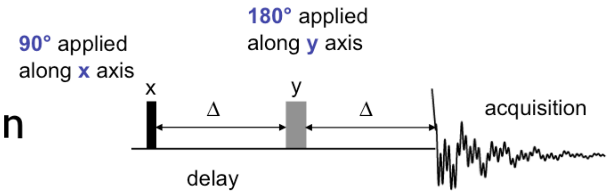
NMR Spectroscopy

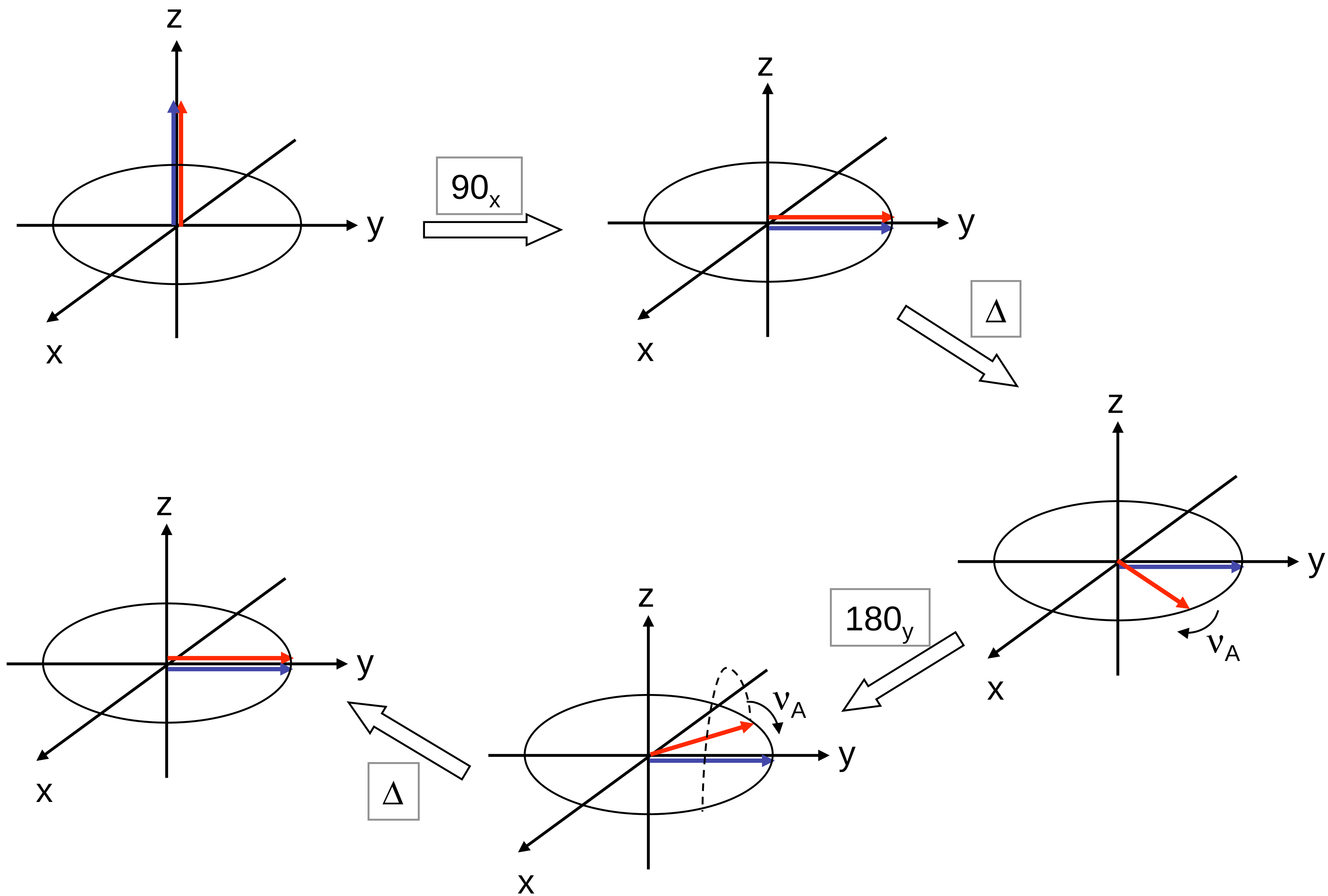
The basic spin-echo pulse sequence



NMR Spectroscopy

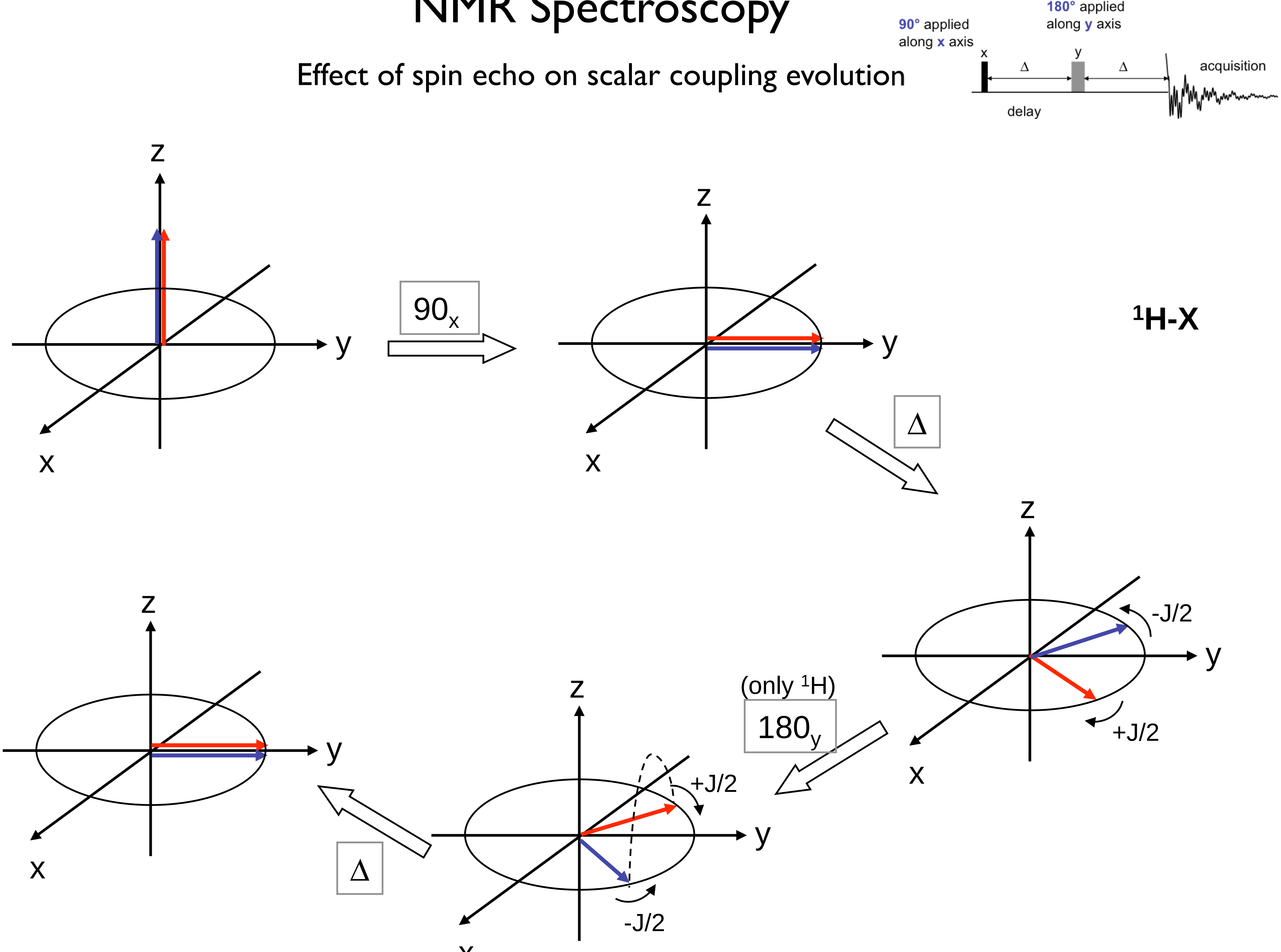
Effect of spin echo on chemical shift evolution





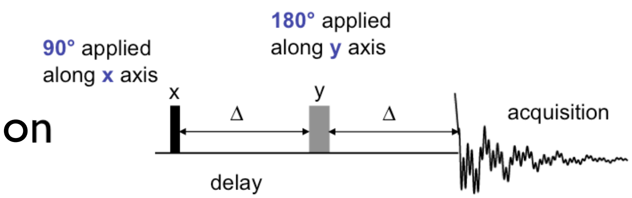
NMR Spectroscopy

Effect of spin echo on scalar coupling evolution

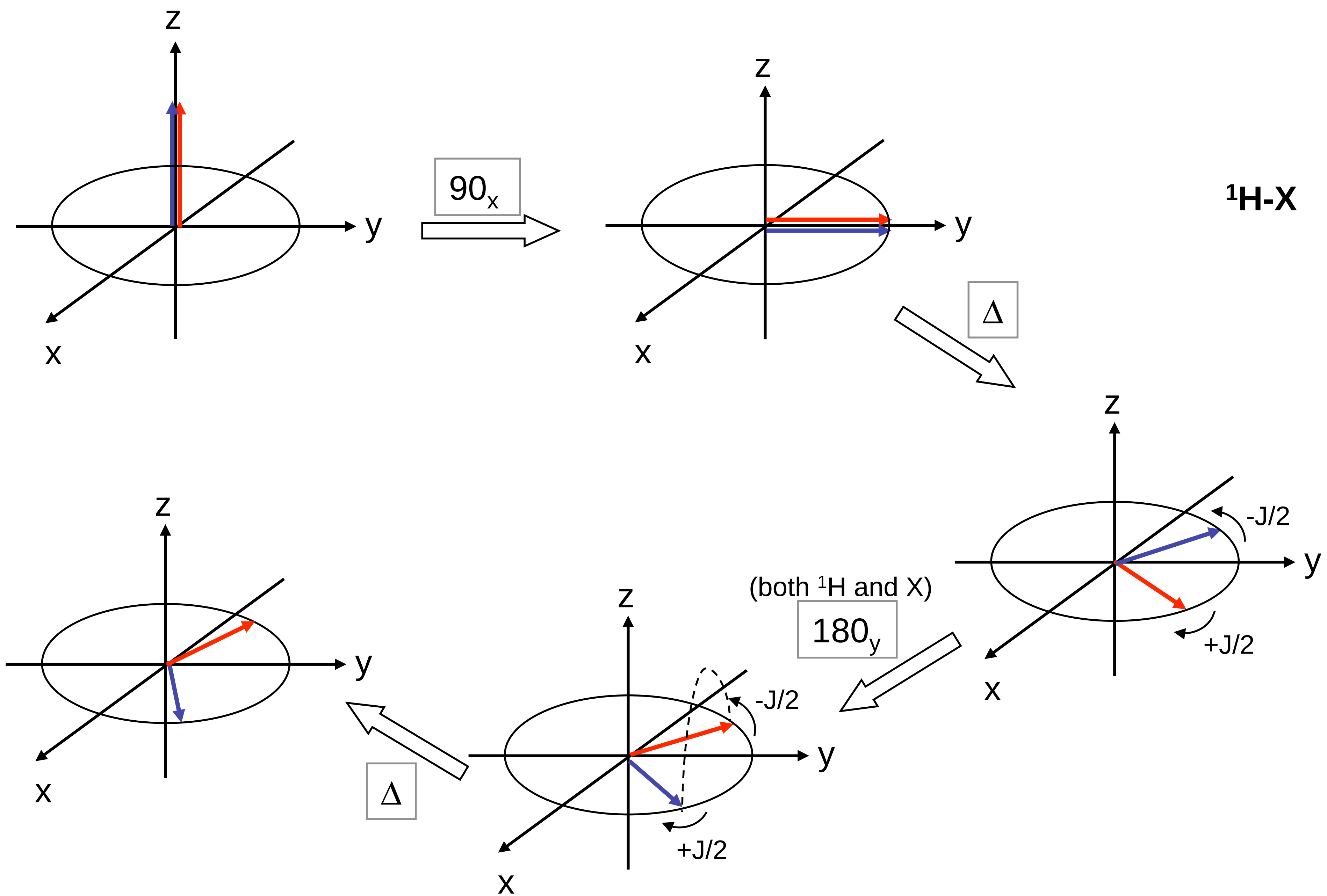


NMR Spectroscopy

Effect of spin echo on scalar coupling evolution

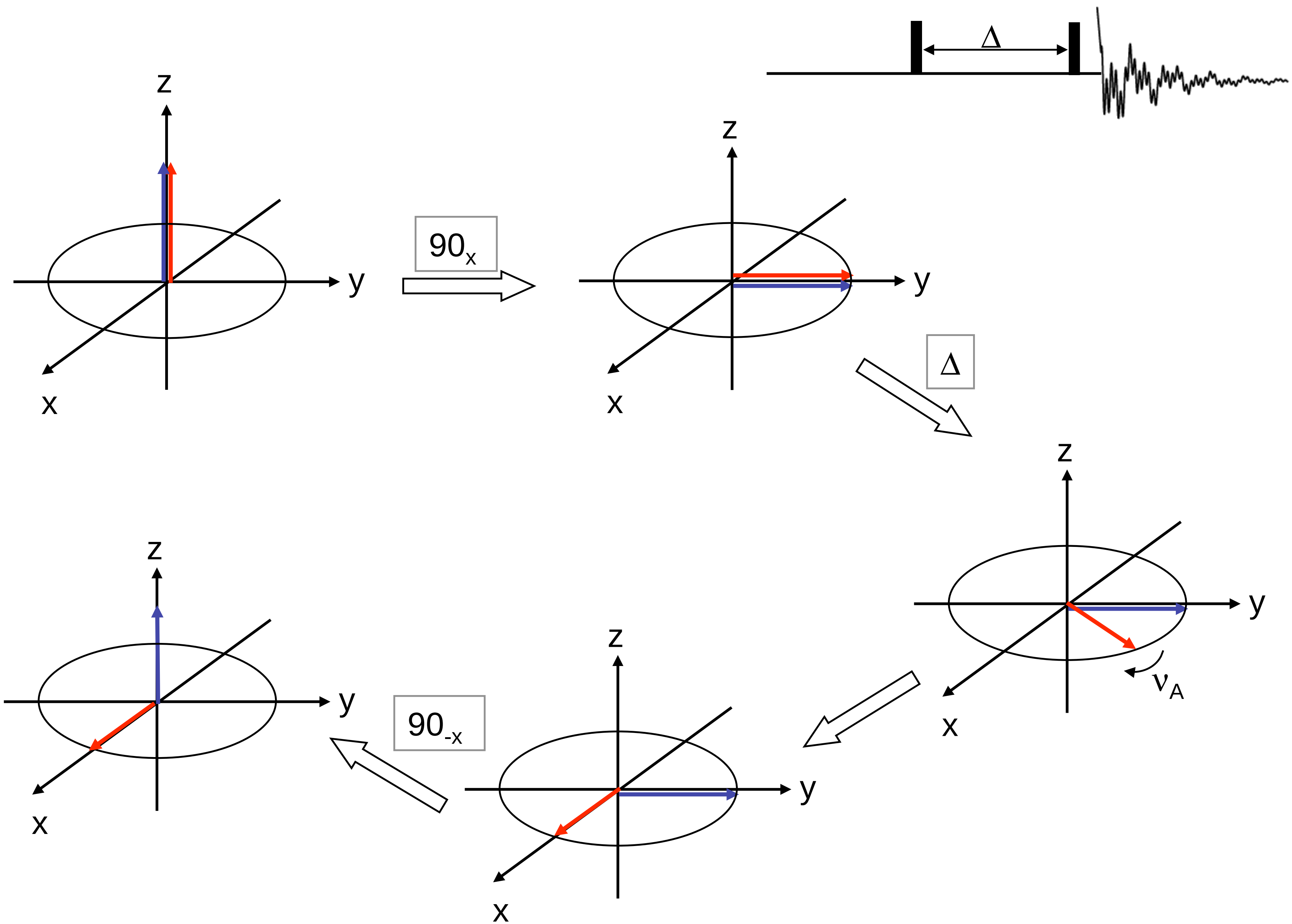


^{1}H-X



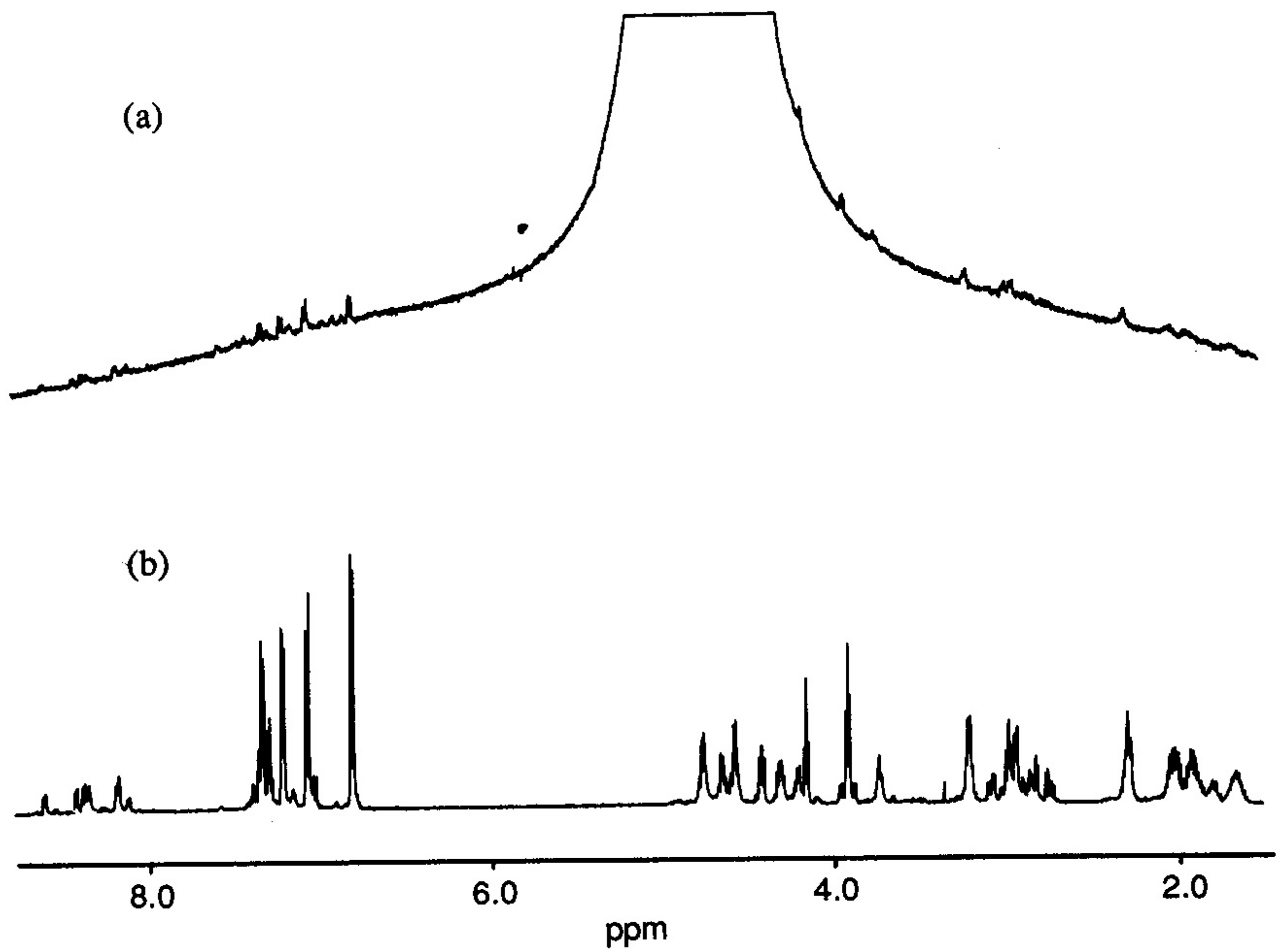
NMR Spectroscopy

Water suppression by the Jump and Return method



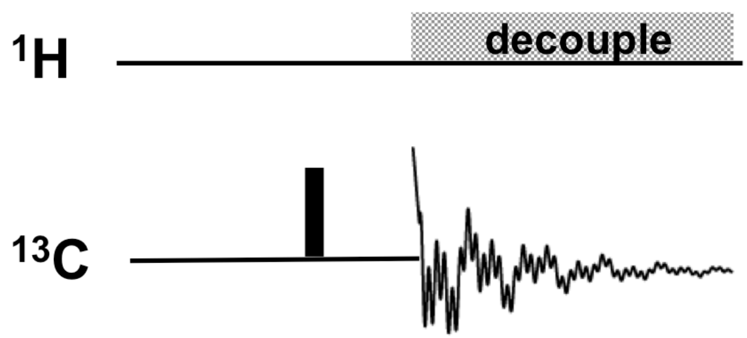
NMR Spectroscopy

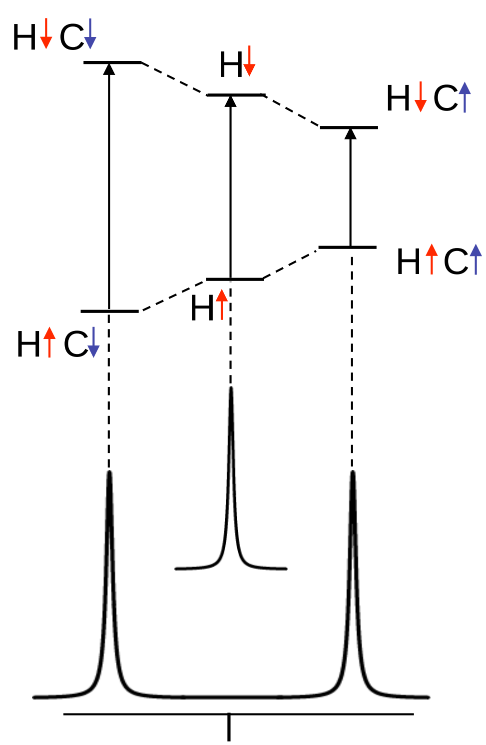
Water suppression



NMR Spectroscopy

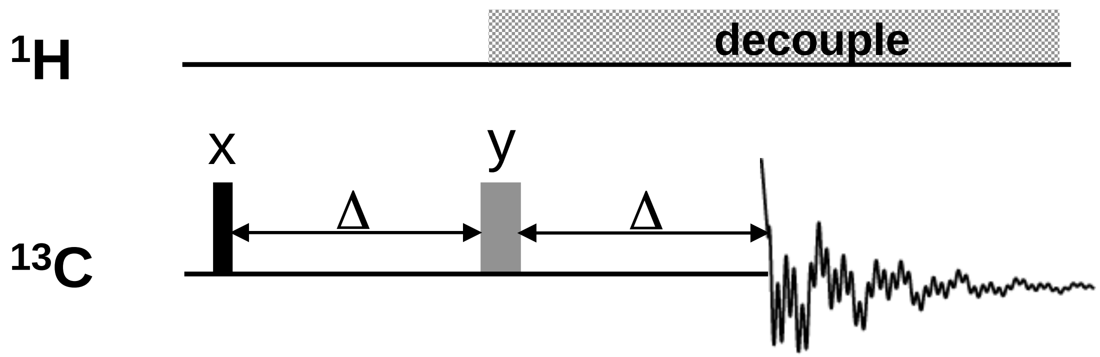
Spin decoupling





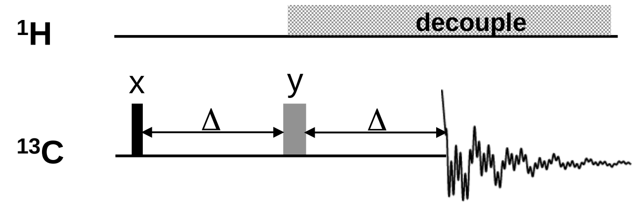
NMR Spectroscopy

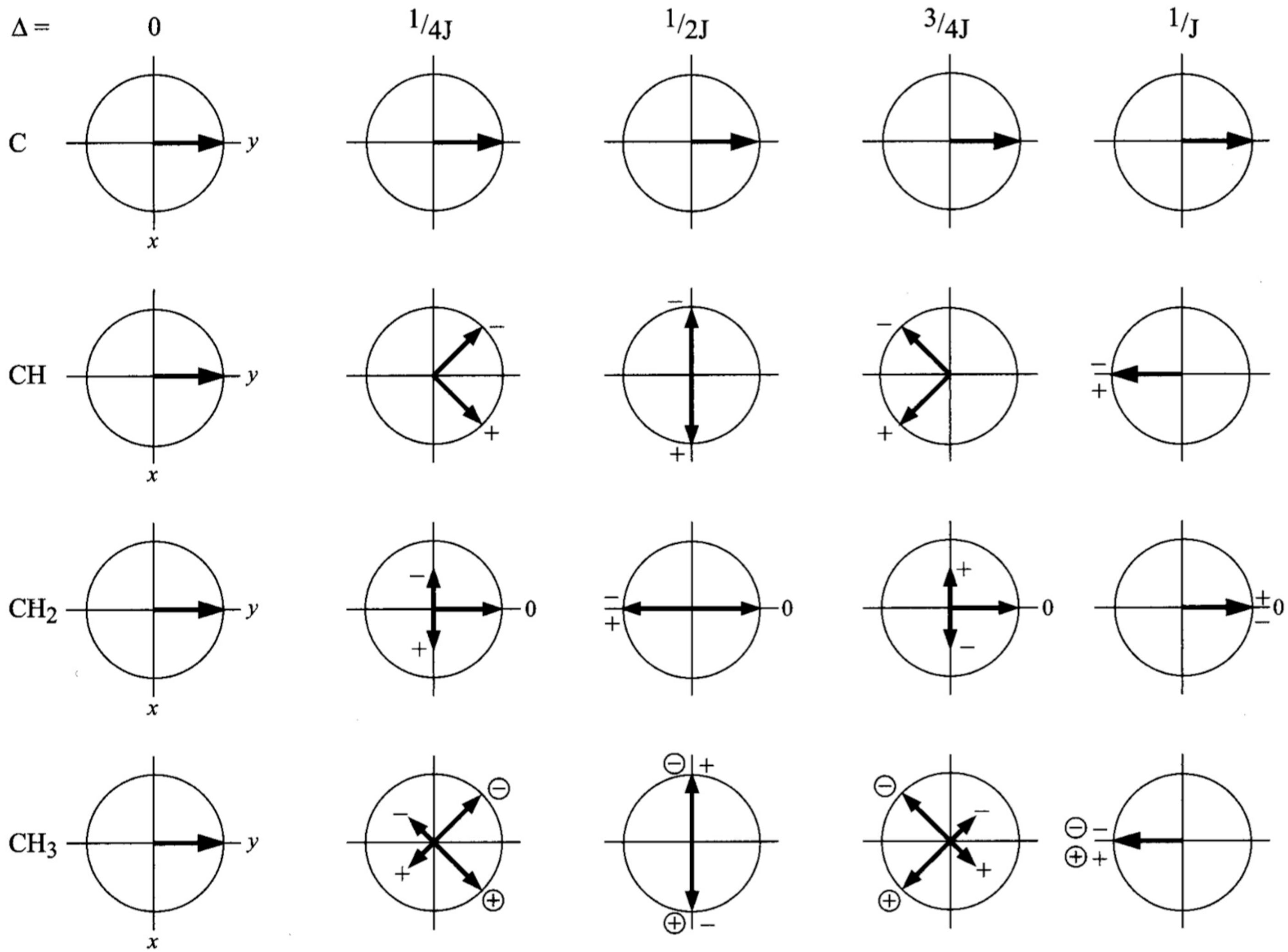
The J-modulated spin echo



NMR Spectroscopy

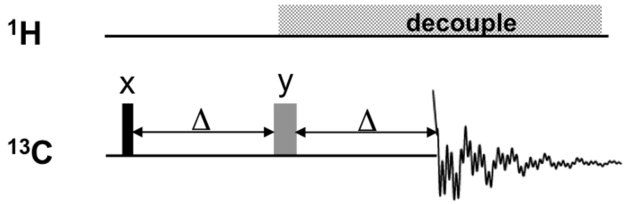
The J-modulated spin echo





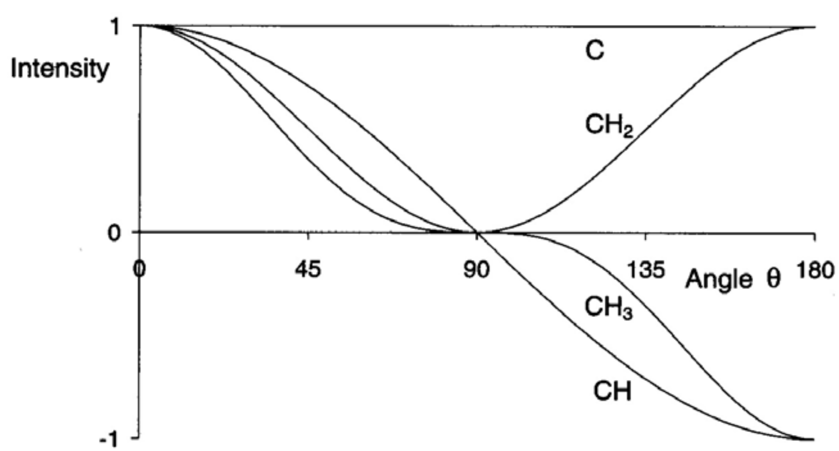
NMR Spectroscopy

The J-modulated spin echo



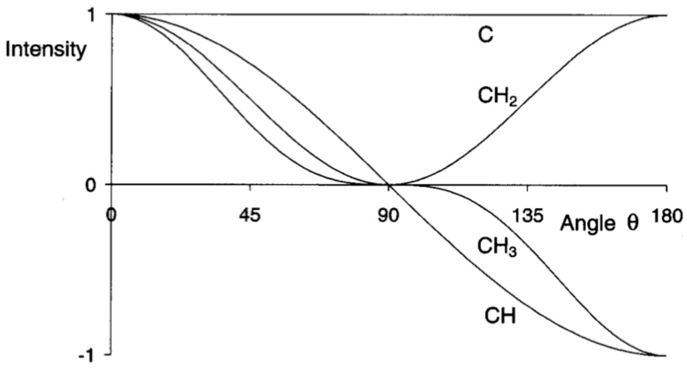
If θ **$=180J\Delta$** degrees

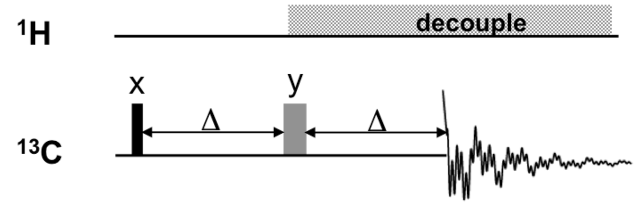
C:	$I=1$
CH:	$I \propto \cos\theta$
CH_2:	$I \propto \cos^2\theta$
CH_3:	$I \propto \cos^3\theta$

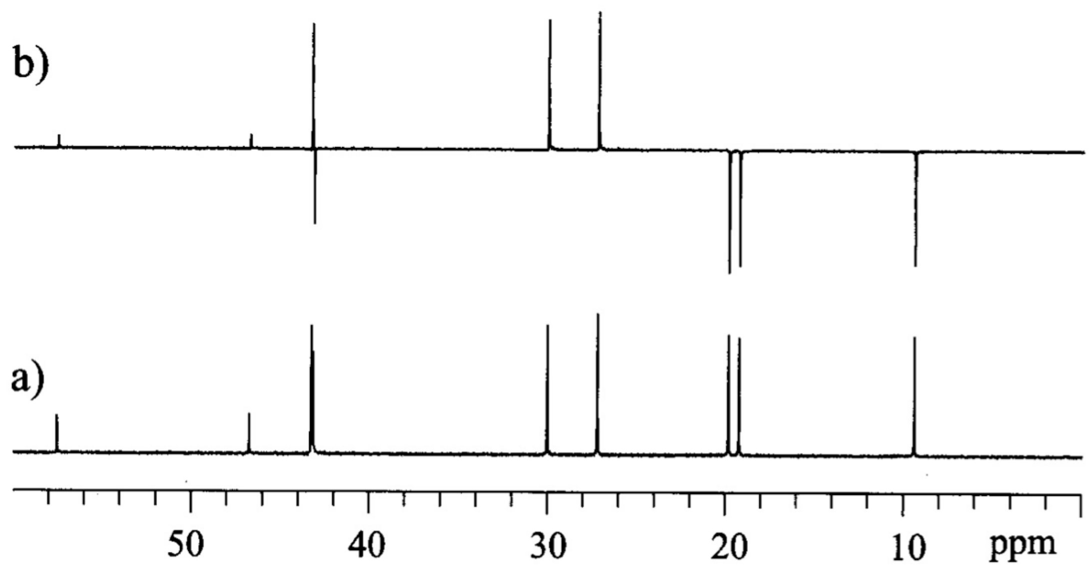


NMR Spectroscopy

The J-modulated spin echo







Δ=1/J

^{13}C (ppm)

NMR Spectroscopy

Sensitivity enhancement

NMR has poor sensitivity compared to other analytical techniques

The intrinsic sensitivity depends upon the gyromagnetic ratio, γ

A greater γ contributes to:

- a high resonant frequency- large transition energy difference- greater Boltzmann population difference
- high magnetic moment and hence a stronger signal
- high rate of precession which induces a greater signal in the detection coil

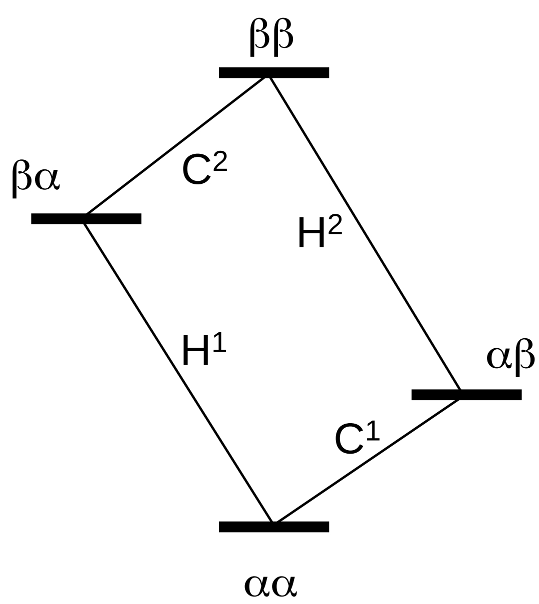
$$\left.\begin{array}{l}\text{So, the strength of NMR signal is proportional to } \gamma^3 \\ \\ \text{Noise increases a square-root of observed frequency}\end{array}\right\} \mathbf{S/N} \propto \gamma^{5/2}$$

$$\frac{S}{N} \propto T^{-1} B_0^{3/2} \gamma_{exc} \gamma_{obs}^{3/2} T_2^* (NS)^{1/2}$$

NMR Spectroscopy

Sensitivity enhancement by polarization transfer

Signal sensitivity enhancement by transferring the greater population differences of high-γ spins onto their spin-coupled low-γ partners.

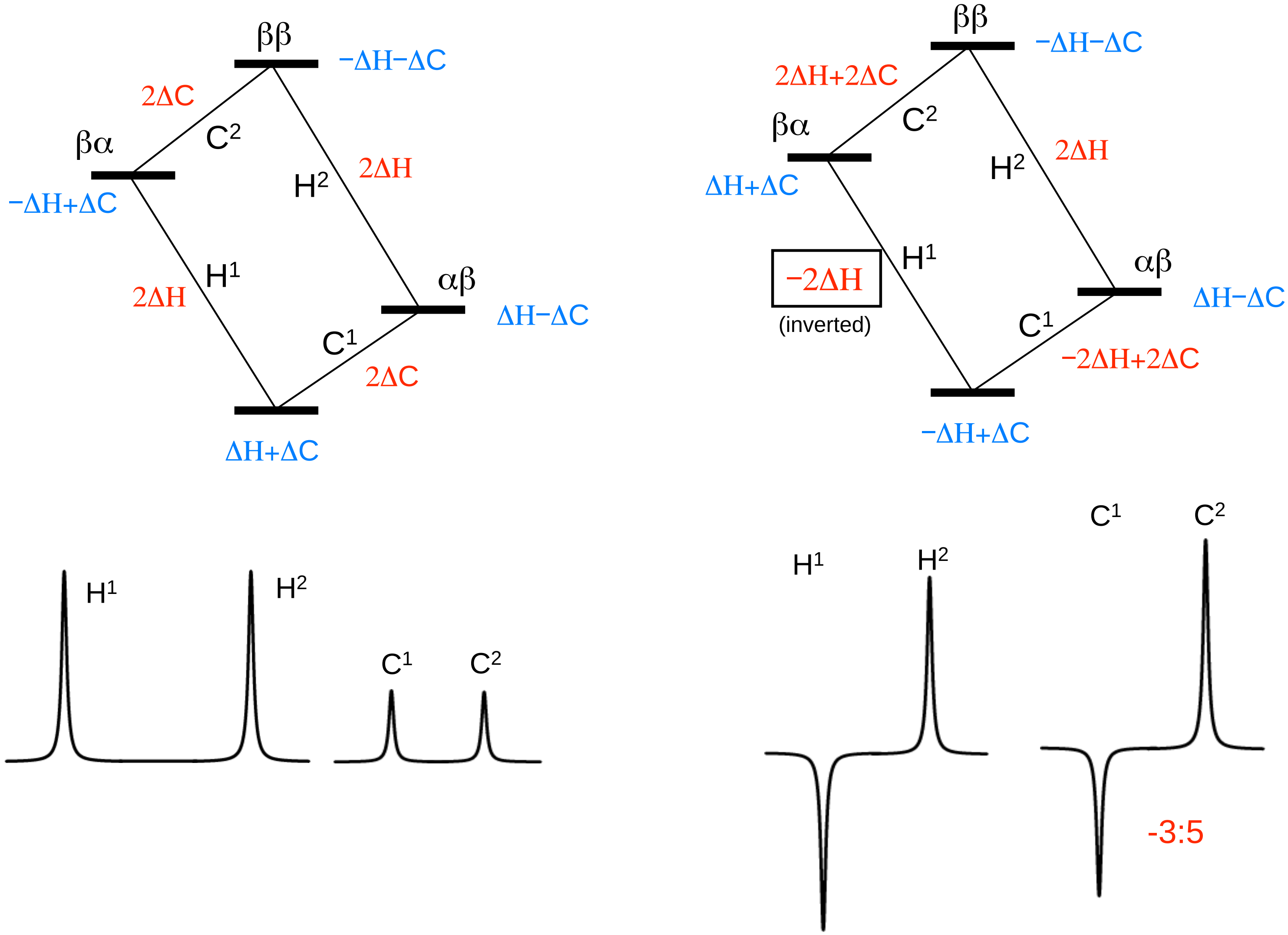


^{1}H-^{13}C spin pair

NMR Spectroscopy

Sensitivity enhancement by polarization transfer

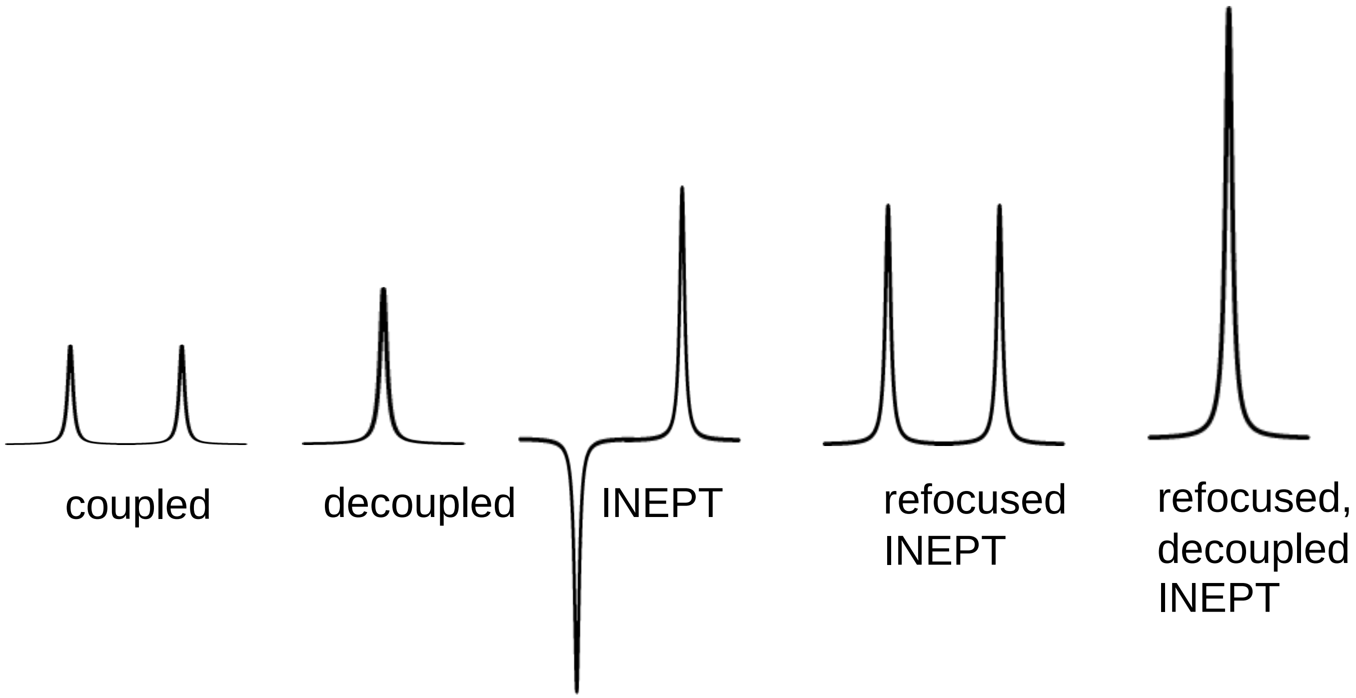
Signal sensitivity enhancement by transferring the greater population differences of high-γ spins onto their spin-coupled low-γ partners.



NMR Spectroscopy

Sensitivity enhancement by polarization transfer

Signal sensitivity enhancement by transferring the greater population differences of high-γ spins onto their spin-coupled low-γ partners.



NMR Spectroscopy

Relaxation

When perturbed, the nuclear spins need to relax to return to their equilibrium distribution

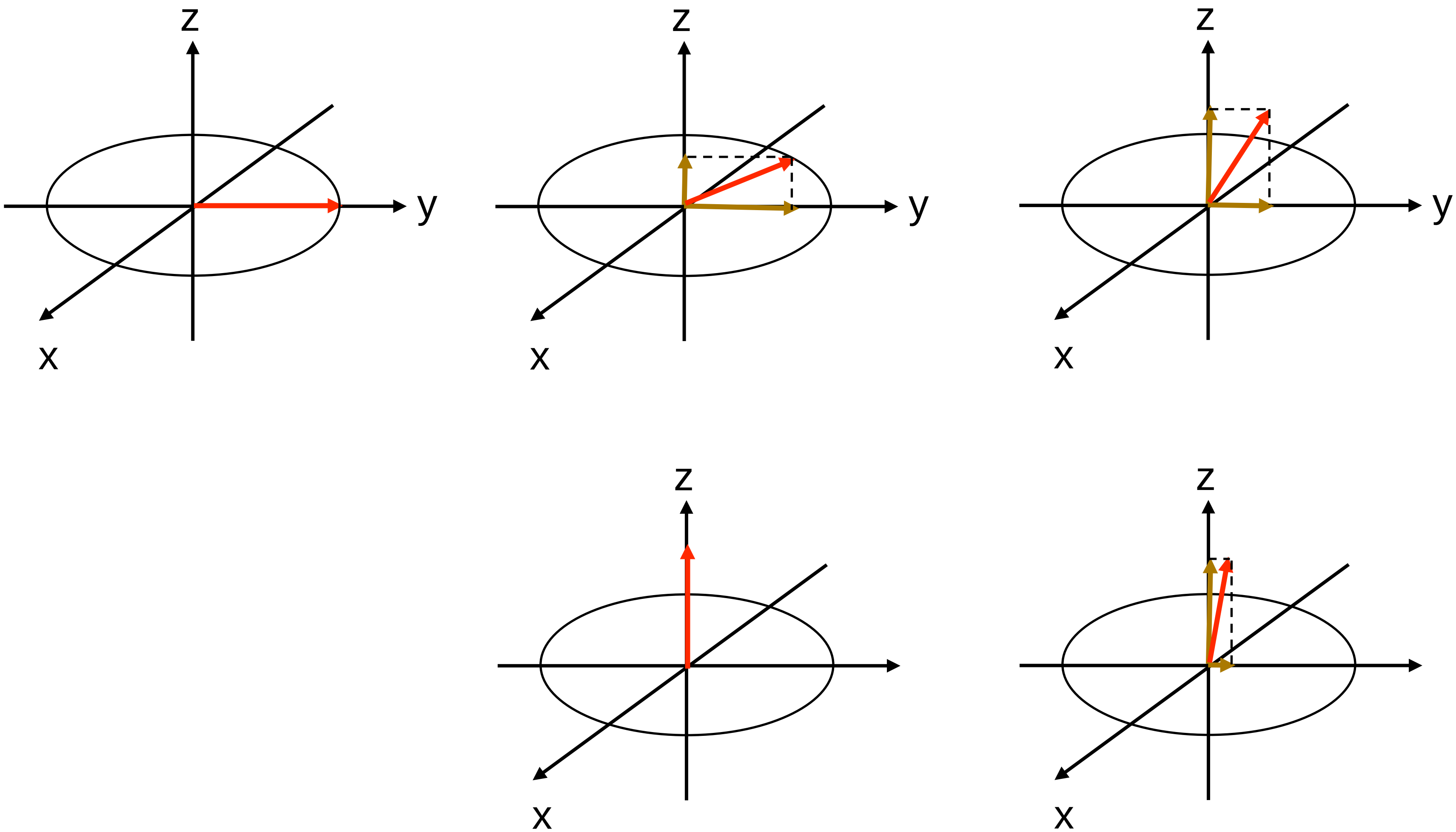
E.g. when the sample is put into a magnet, the Boltzmann distribution of spins among the energy levels changes due to a change in the energy of the various levels

E.g. after applying electromagnetic radiation, which induces transitions between energy levels, the system returns to its equilibrium

This process is called relaxation

NMR Spectroscopy

Longitudinal Relaxation: Establishing Equilibrium



NMR Spectroscopy

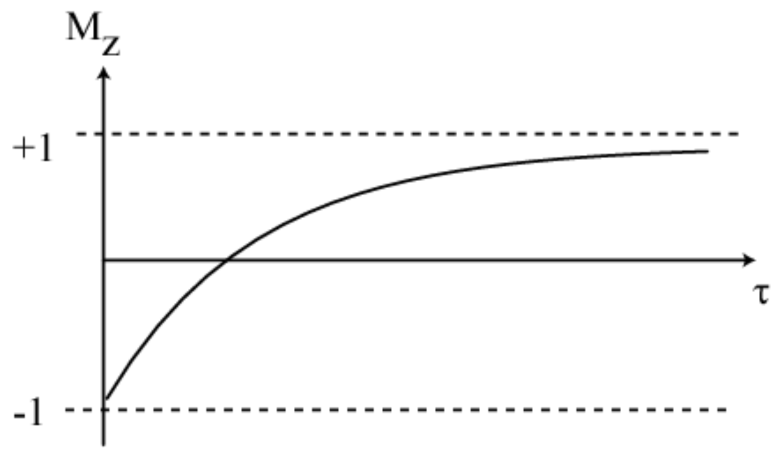
Longitudinal Relaxation: Establishing Equilibrium

Recovery of the z-magnetization follows exponential behavior

$$\frac{dM_z}{dt} = \frac{(M_0 - M_z)}{T_1}$$

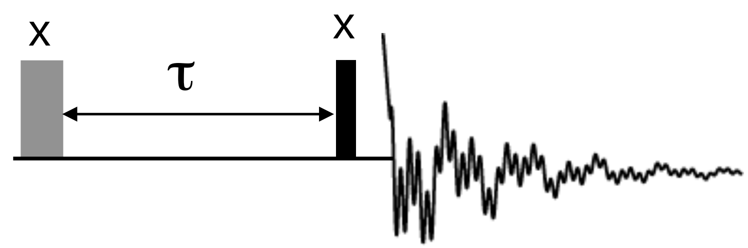
$$M_z = M_0\,(1-2e^{-t/T1})$$

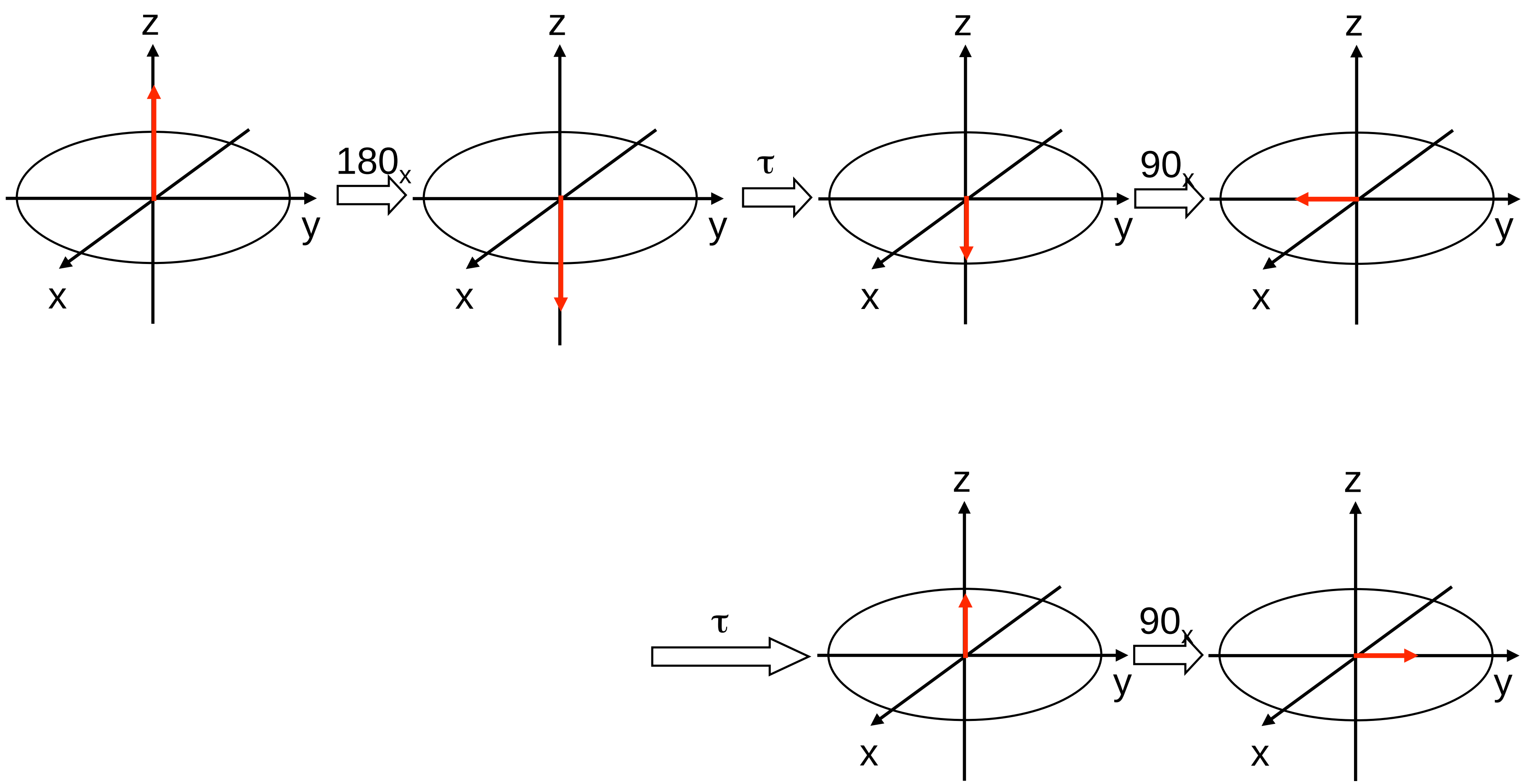
where T_1 is the longitudinal relaxation time



NMR Spectroscopy

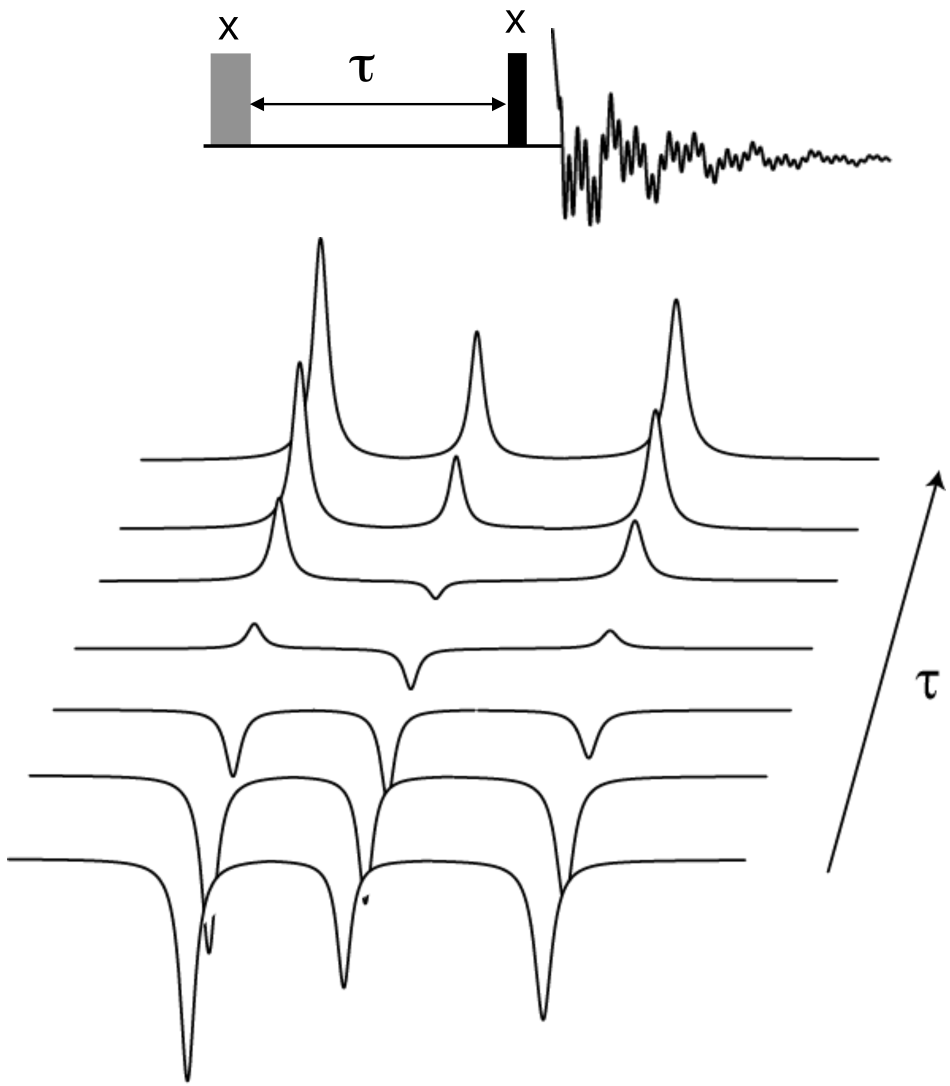
Longitudinal Relaxation: Measurement





NMR Spectroscopy

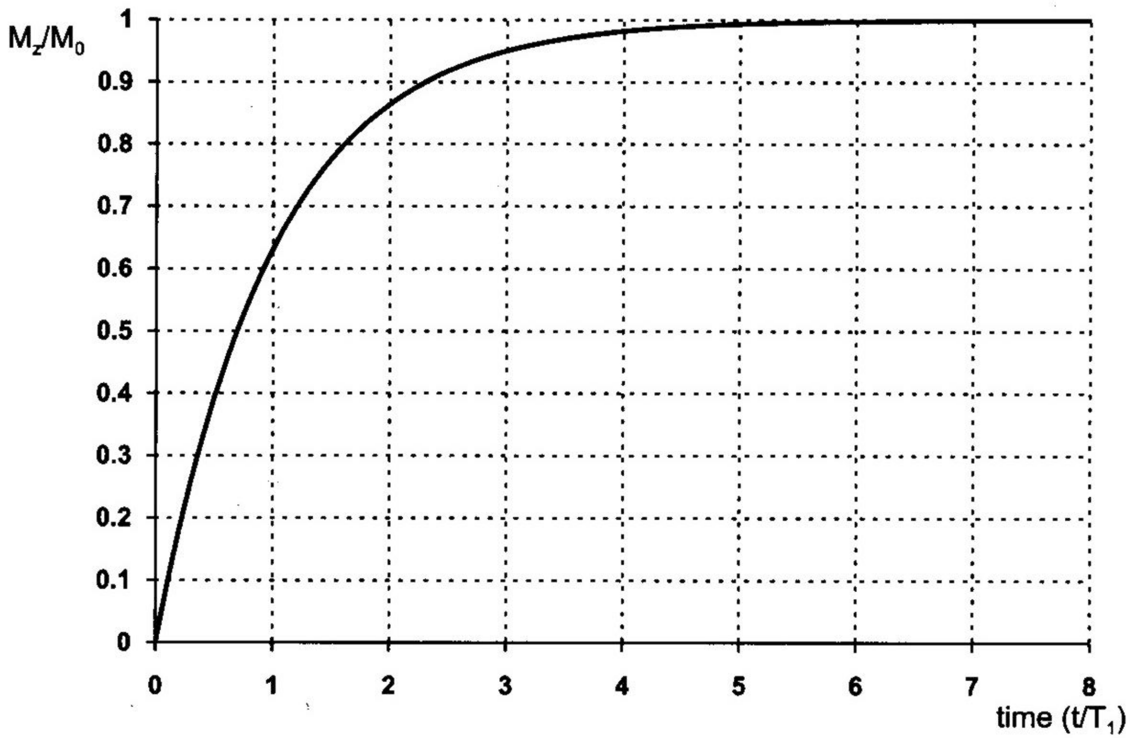
Longitudinal Relaxation: Measurement



NMR Spectroscopy

Longitudinal Relaxation: Exponential growth

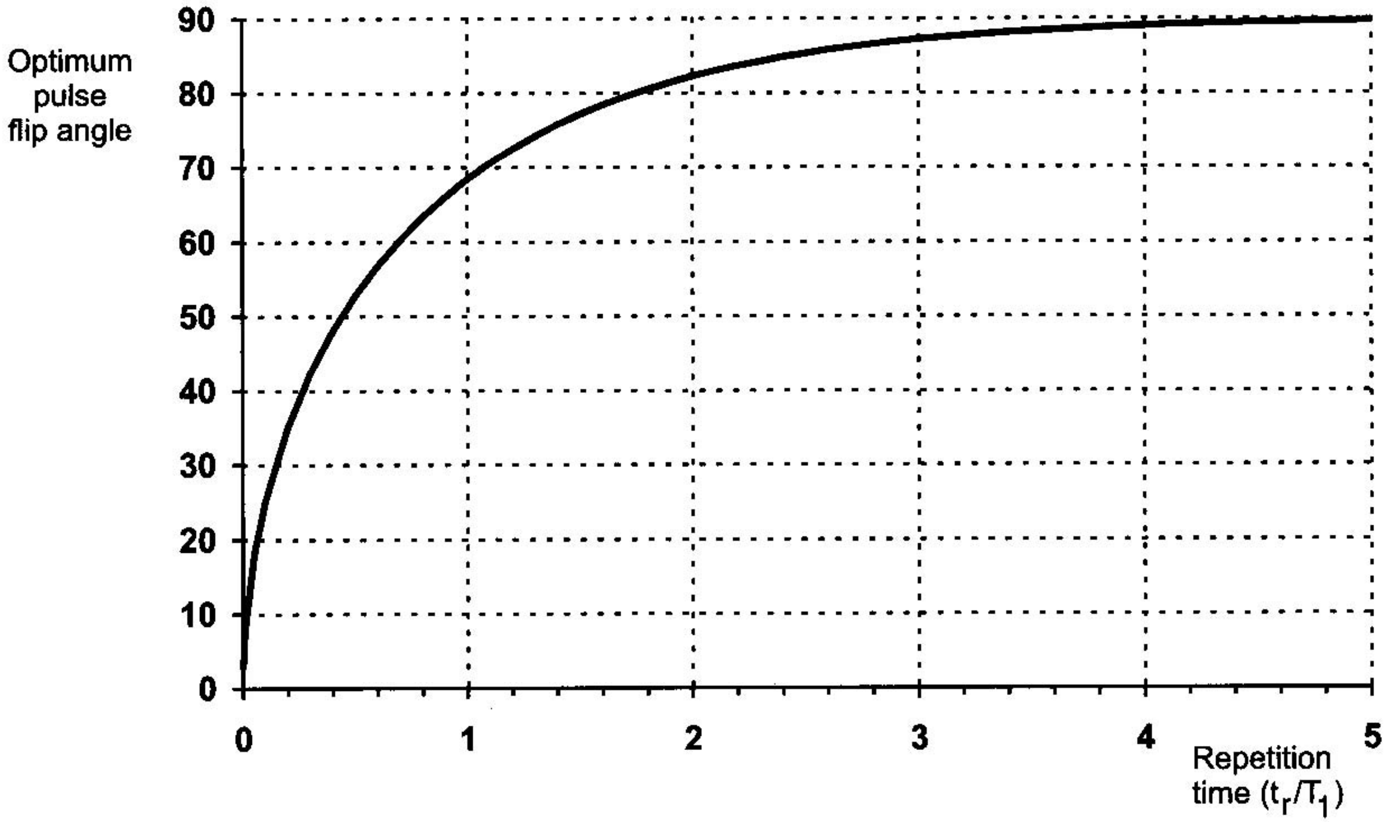
$$M_z = M_0 \left(1 - 2e^{-t/T1}\right)$$



By the end of $5T_1$ sec, the magnetization has recovered by 99.33%

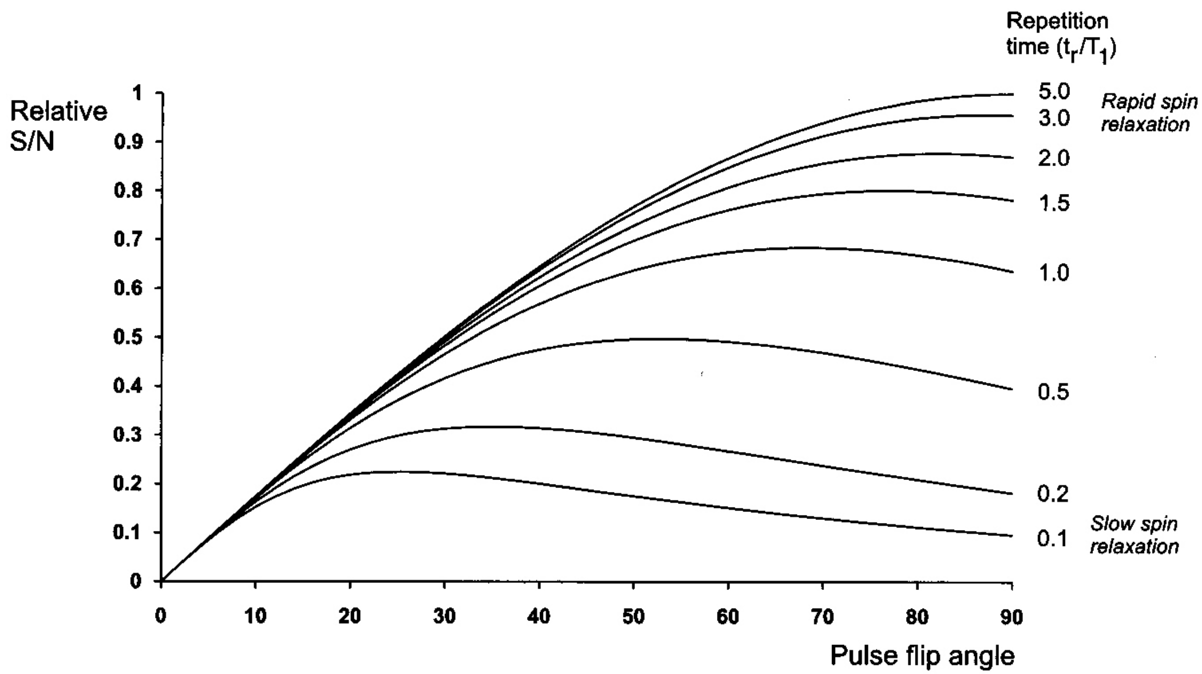
NMR Spectroscopy

Longitudinal Relaxation: optimizing sensitivity



NMR Spectroscopy

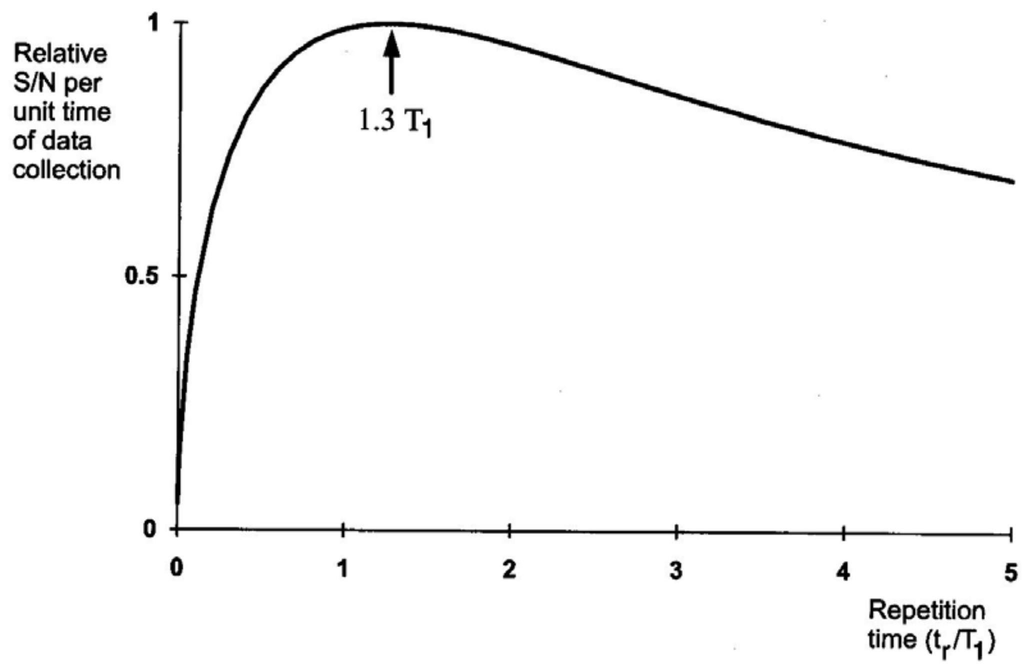
Longitudinal Relaxation: optimizing sensitivity



NMR Spectroscopy

Longitudinal Relaxation: optimizing sensitivity

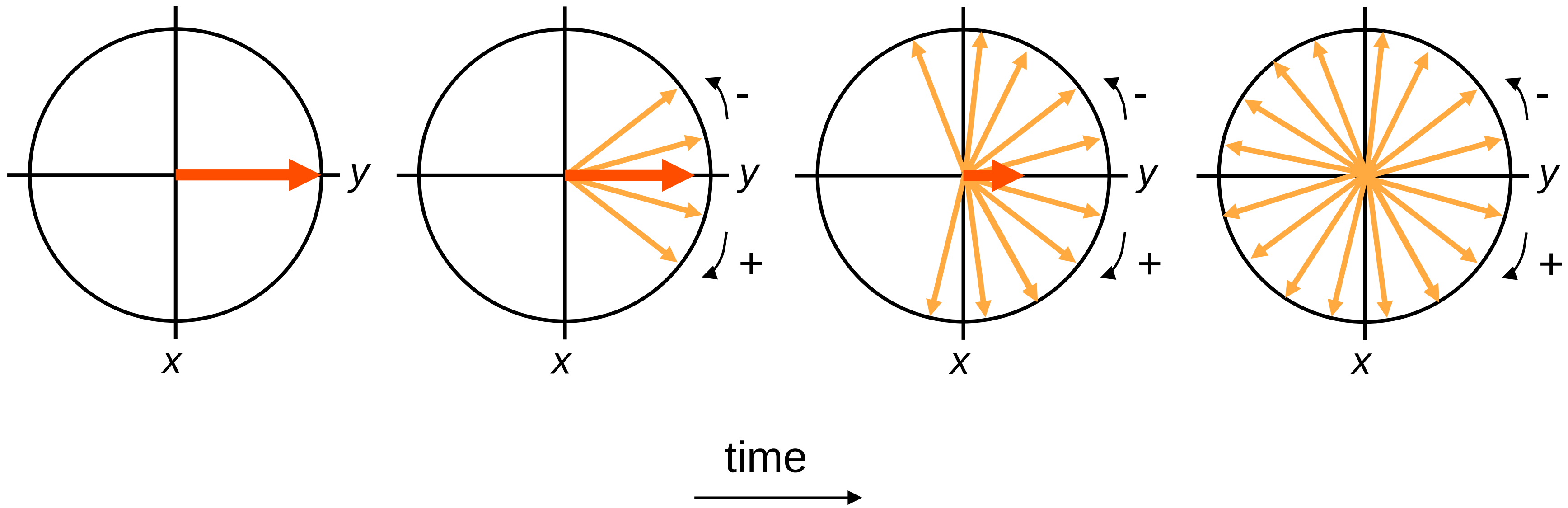
optimum pulse repetition time when using 90º



Quantitative measurements and integration

NMR Spectroscopy

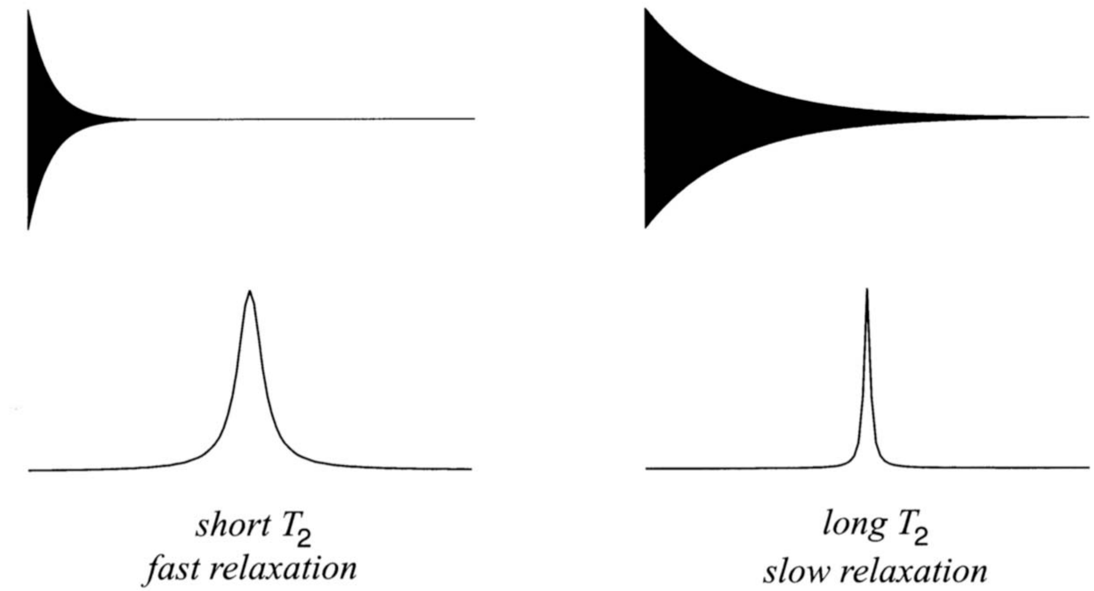
Transverse Relaxation: magnetization loss in the x-y plane



$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_{2(\Delta B_o)}}$$

NMR Spectroscopy

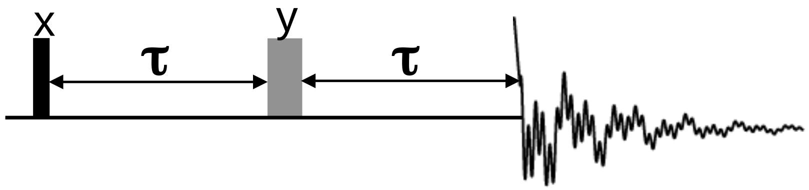
Transverse Relaxation: magnetization loss in the x-y plane

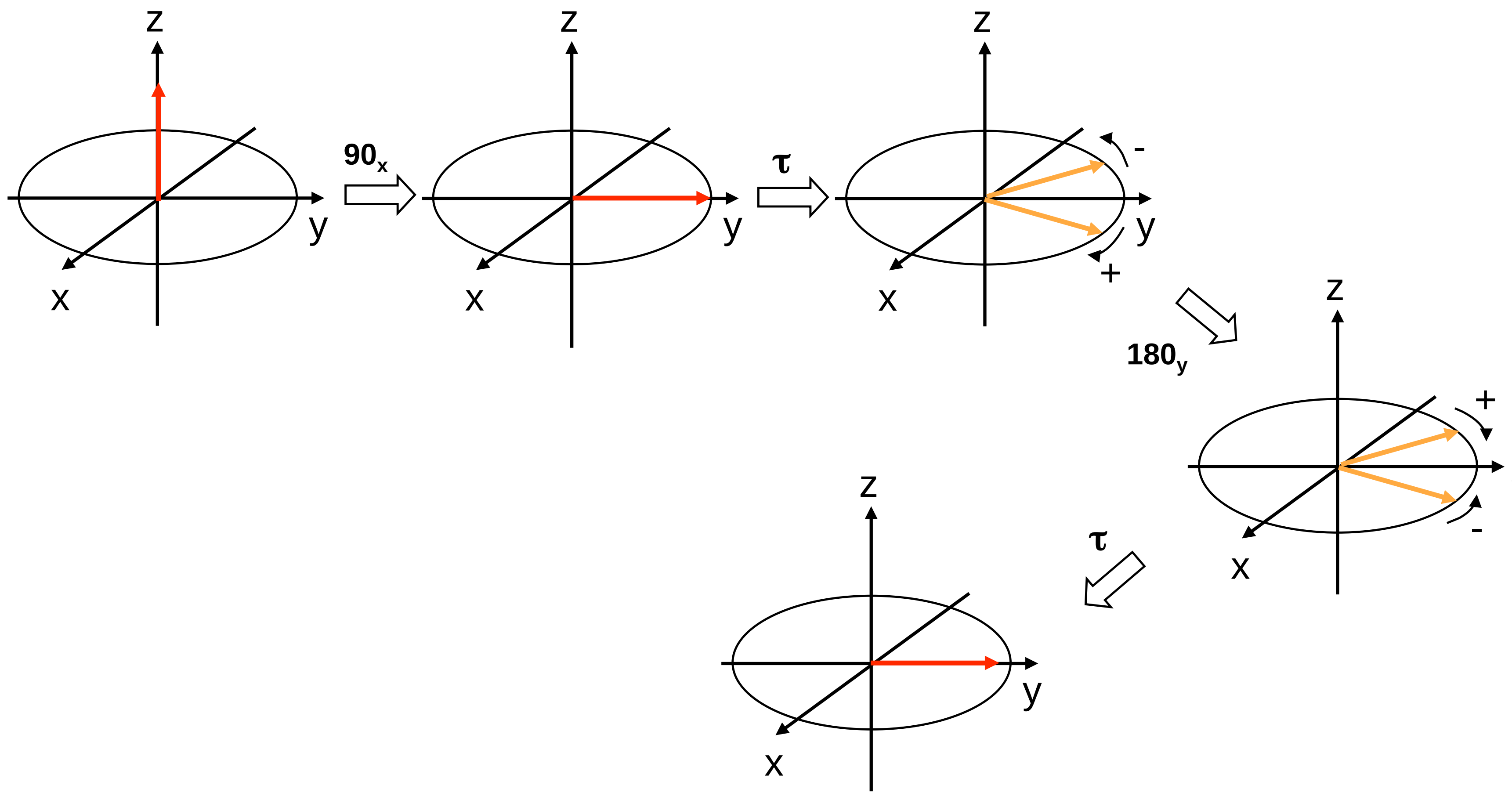


$$\Delta\nu = \frac{1}{\pi\, T_2^*}$$

NMR Spectroscopy

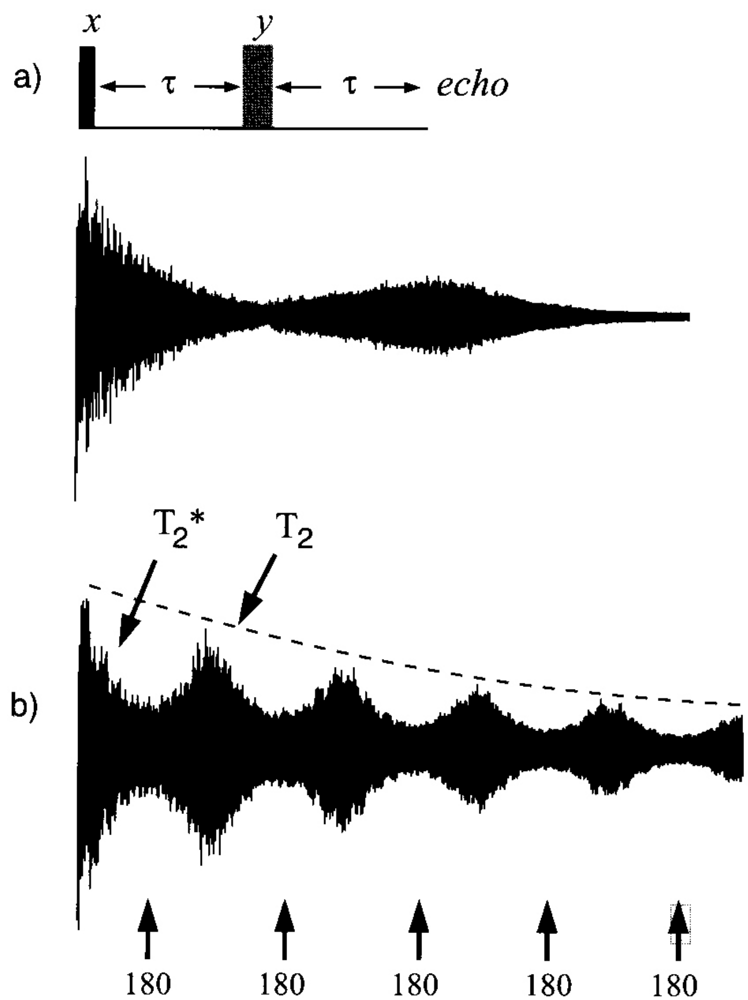
Transverse Relaxation: Measurement





NMR Spectroscopy

Transverse Relaxation: Measurement



NMR Spectroscopy

T1 *vs* T2 Relaxation

$\boxed{T_1 \geq T_2}$

For small molecules, $T_1 \approx T_2$

For large molecules, $T_1 >> T_2$

Longitudinal relaxation causes loss of energy from the spins (enthalpic)

Transverse relaxation occurs by mutual swapping of energy between spins (entropic)

NMR Spectroscopy

Relaxation mechanisms

Nuclear spin relaxation is not a spontaneous process; it requires stimulation by suitable **fluctuating fields** to induce the necessary spin transitions

Two main mechanisms

Dipole-dipole

Chemical shift anisotropy

NMR Spectroscopy

Relaxation mechanisms

Longitudinal relaxation requires a time-dependent magnetic field fluctuating at the Larmor frequency

The time-dependence originates in the motions of the molecule (vibration, rotation, diffusion etc)

Molecules in solution "tumble". This "tumbling" can be characterized by a rotational correlation time τ_c

τ_c is the time needed for the rms deflection of the molecules to be ~ 1 radian (60°)

NMR Spectroscopy

Spectral density function

Rotational diffusion in solution occurs at a range of frequencies

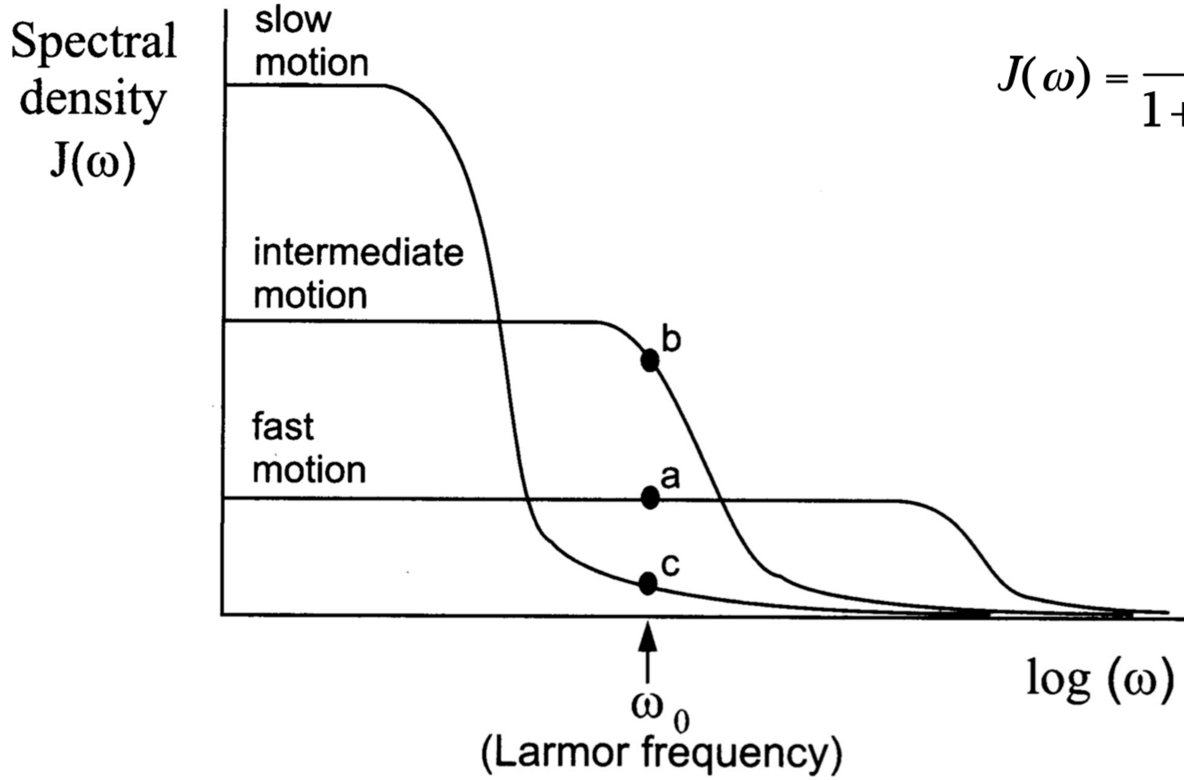
$1/\tau_c$ ~ rms rotational frequency (radians s^{-1})

The probability function of finding motions at a given angular frequency ω can be described by the spectral density function $J(\omega)$

$$J(\omega) = \frac{2\tau_c}{1+(\omega\tau_c)^2}$$

NMR Spectroscopy

Spectral density function



$$J(\omega) = \frac{2\tau_c}{1+(\omega\tau_c)^2}$$

Frequency distribution of the fluctuating magnetic fields

NMR Spectroscopy

Spectral density function: Longitudinal relaxation

Spins are relaxed by local fields fluctuating at the Larmor frequency ω_0

So, the relaxation rate (R1) will be proportional to the $J(\omega_0)$

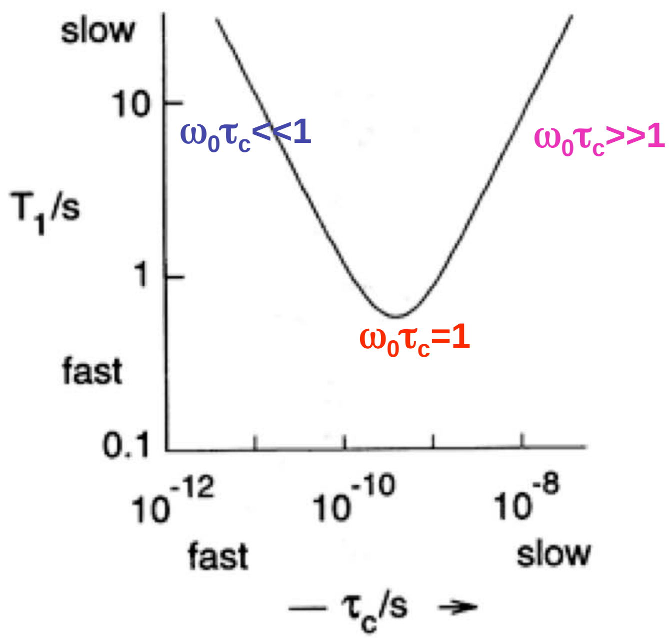
$$1/T_1 = R_1 = \gamma^2 \langle B^2 \rangle J(\omega_0)$$

Knowing the form of $J(\omega)$ we can predict the dependence of the spin-lattice relaxation time (T1=1/R1) on the correlation time τ_c for a given NMR frequency ω_0

$\omega_0\tau_c=1$, $J(\omega_0) = \tau_c = 1/\omega_0$
and T_1 is minimum (R_1 maximum)

$\omega_0\tau_c \ll 1$ (small molecules), $J(\omega_0) \sim 2\tau_c$ and T_1 decreases (R_1 increases) with increasing τ_c (e.g.by decreasing the temperature)

$\omega_0\tau_c \gg 1$ (large molecules), $J(\omega_0) \sim 2/\omega_0^2\tau_c$ and T_1 increases (R_1 decreases) with increasing τ_c (e.g. by decreasing the temperature)



NMR Spectroscopy

Relaxation mechanisms: Dipole-dipole

Nuclei with non-zero quantum numbers have magnetic dipoles

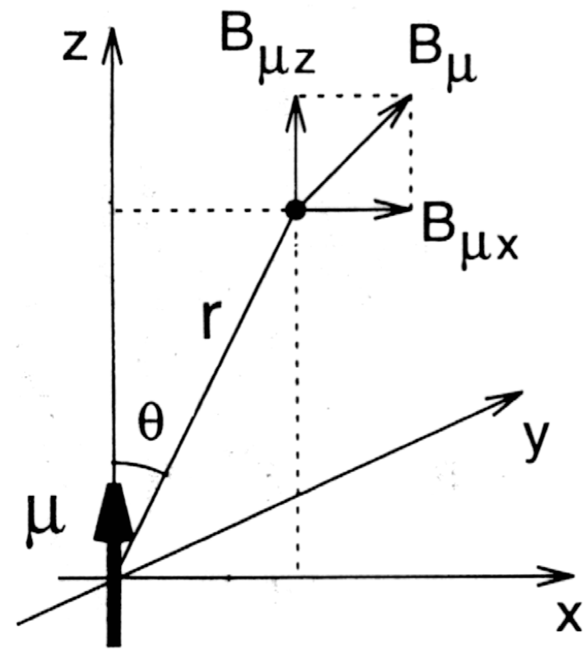
They behave like small magnets and induce small magnetic fields that affect neighboring nuclei

Magnetic field, **B**$_{\mu}$, generated by a magnetic dipole μ

$$B_{\mu x} = \left(\frac{\mu_0}{4\pi}\right)\left(\frac{\mu}{r^3}\right)(3\sin\theta\,\cos\theta)$$

$$B_{\mu y} = 0$$

$$\boxed{B_{\mu z} = \left(\frac{\mu_0}{4\pi}\right)\left(\frac{\mu}{r^3}\right)(3\cos^2\theta - 1)}$$

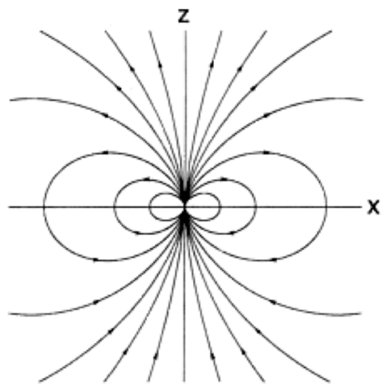


NMR Spectroscopy

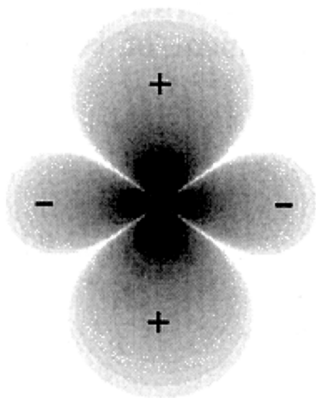
Relaxation mechanisms: Dipole-dipole

Representation of the dipolar magnetic field B_μ, generated by a magnetic dipole μ

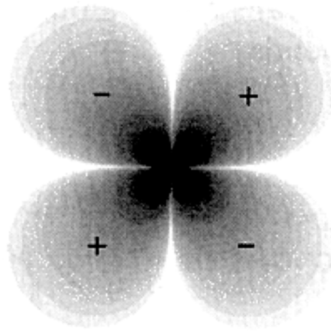
lines of force



density plots



$\mathbf{B_{\mu z}}$



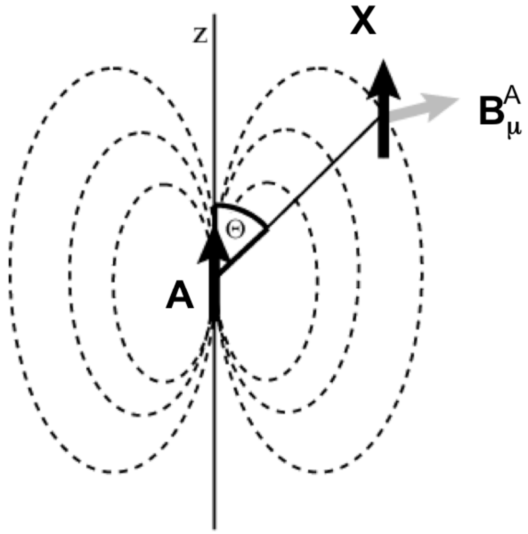
$\mathbf{B_{\mu x}}$

$B_{\mu z}$ is zero for $\theta=\pm 54.7^\circ$ (magic angle)

NMR Spectroscopy

Relaxation mechanisms: Dipole-dipole

The **z** component of their dipole magnetic field will affect the field experienced by the other nucleus and cause splitting



$$B^X = B_0 \pm B^A_{\mu Z}$$

± sign refers to the quantum number of A (±½)

Thus, the splitting in the spectrum of X is

$$J^{heteronuclear}_{dipolar} = K_{AX}(3\cos^2\theta - 1)$$

$$2\pi K_{AX} = \left(\frac{\mu_0}{4\pi}\right)\frac{\hbar\gamma_A\gamma_X}{r^3_{AX}}$$

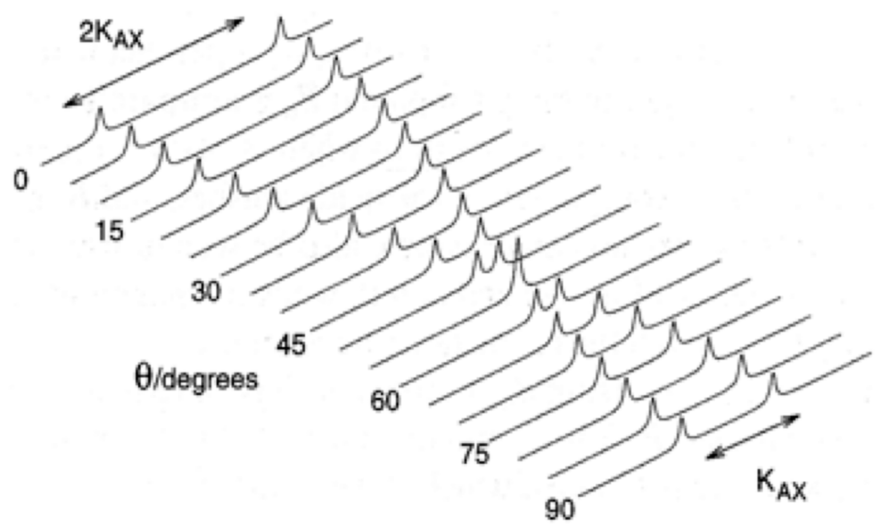
K_{AX} vary with the distance

e.g. K_{CH} is 9000 Hz at 1.5 Å and 30 Hz at 10 Å

NMR Spectroscopy

Relaxation mechanisms: Dipole-dipole

Splitting of the AX spectrum depends on θ



In a crystal with fixed distances and angles the dipolar splitting vary with the crystal orientation with respect to the external magnetic field

NMR Spectroscopy

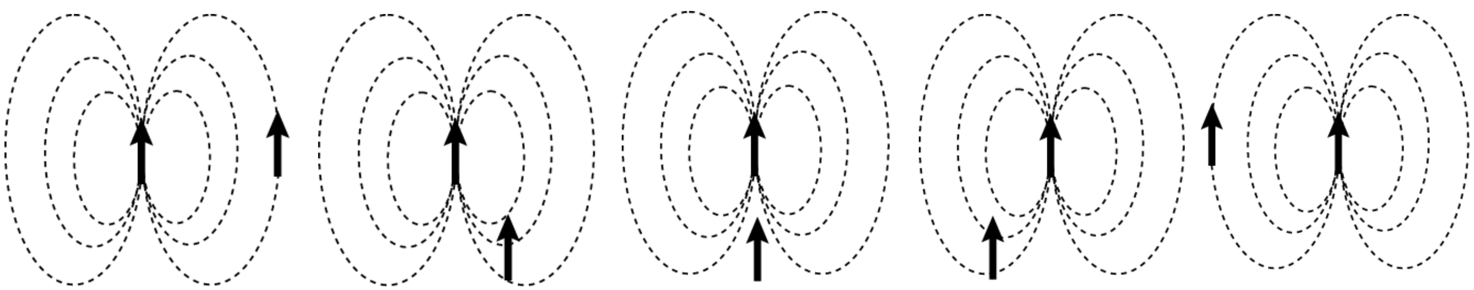
Relaxation mechanisms: Dipole-dipole

Molecules in liquids rotate, "tumble" rapidly with typical frequencies between 10^{12} to 10^{8} Hz for small molecules and proteins, respectively.

Those frequencies are much larger than typical dipolar couplings (10^{5} Hz)

The angular part of the dipolar splitting is averaged over all possible orientation to 0

Although they are not directly observed in solution, dipolar couplings play an important role in spin relaxation



The local field experienced at one nucleus as a result of its neighbor will fluctuate as the molecule tumbles

NMR Spectroscopy

Relaxation mechanisms: Dipole-dipole

$$R_1 = \frac{1}{T_1} = \left(\frac{\mu_o}{4\pi}\right)^2 \frac{\gamma_I^2 \gamma_S^2 \hbar^2 \tau_c}{r_{IS}^6}$$

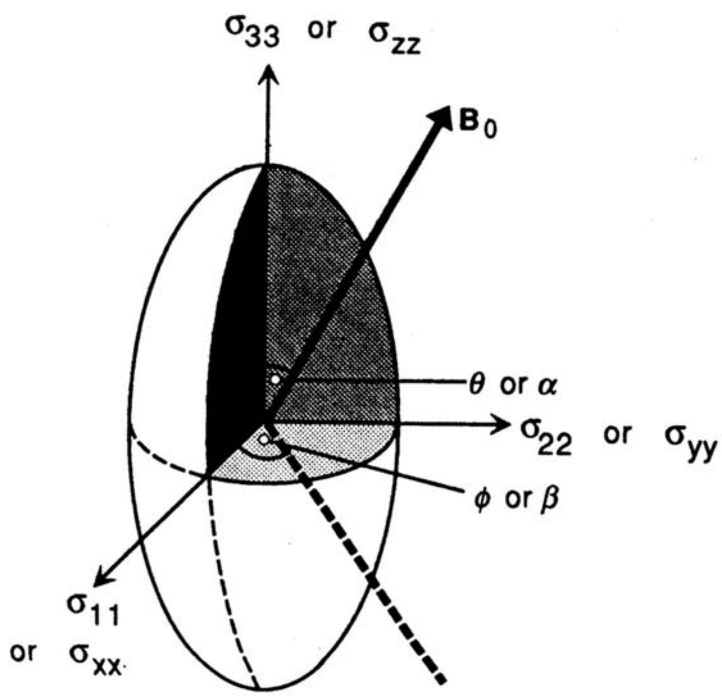
R1 depend of the gyromagnetic ratio of the nuclei (e.g. H-H relaxation more efficient than C-H)

NMR Spectroscopy

Relaxation mechanisms: Chemical shift anisotropy

The distribution of the electrons about the nucleus is non-sperical- thus, the magnitude of the shielding depends on the relative orientation of the nucleus with respect to the static field.

As the molecule tumbles, it creates a fluctuating magnetic field



$$R_1 = \frac{1}{T_{1(CSA)}} = \frac{2}{15}\gamma^2 B_0^2 (\Delta\sigma^2)\tau_c$$

NMR Spectroscopy

Nuclear Overhauser Effect (NOE)

NOE: change in intensity of one resonance when the spin transitions of another are perturbed from their equilibrium populations

perturbation: saturation or inversion

The two spins should "communicate" through dipole-dipole interaction

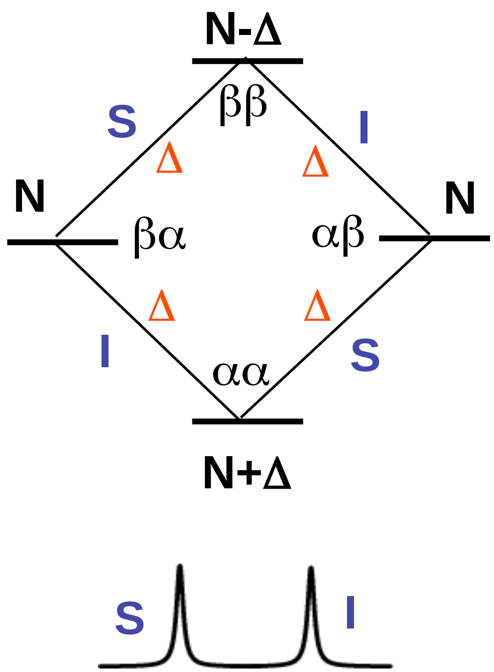
$$\eta_I\{S\} = \frac{I - I_o}{I_0} \times 100\ (\%)$$

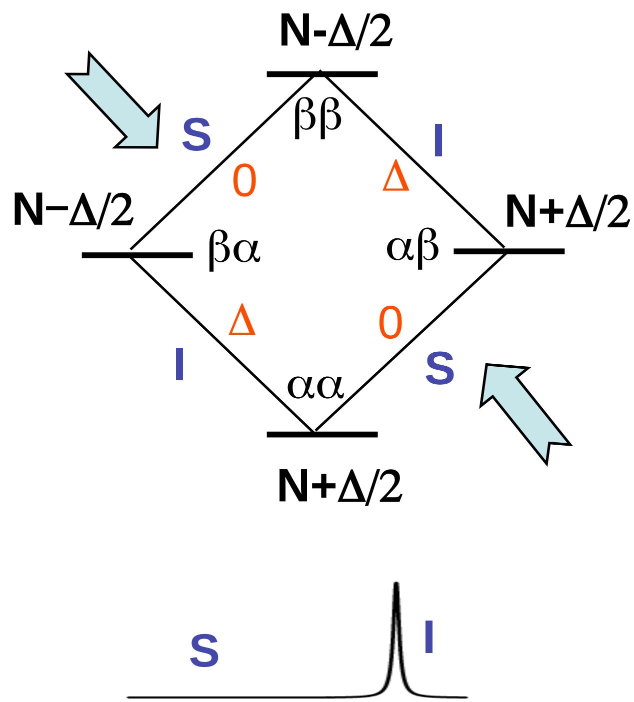
NOE is observed for spin I when spin S is perturbed

NMR Spectroscopy

Nuclear Overhauser Effect (NOE)

Origin of the NOE

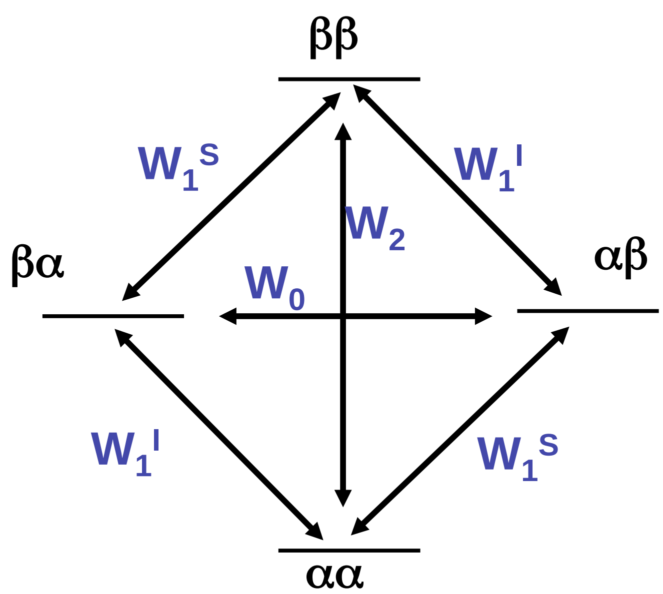




NMR Spectroscopy

Nuclear Overhauser Effect (NOE)

Six possible transitions in a two-spin system

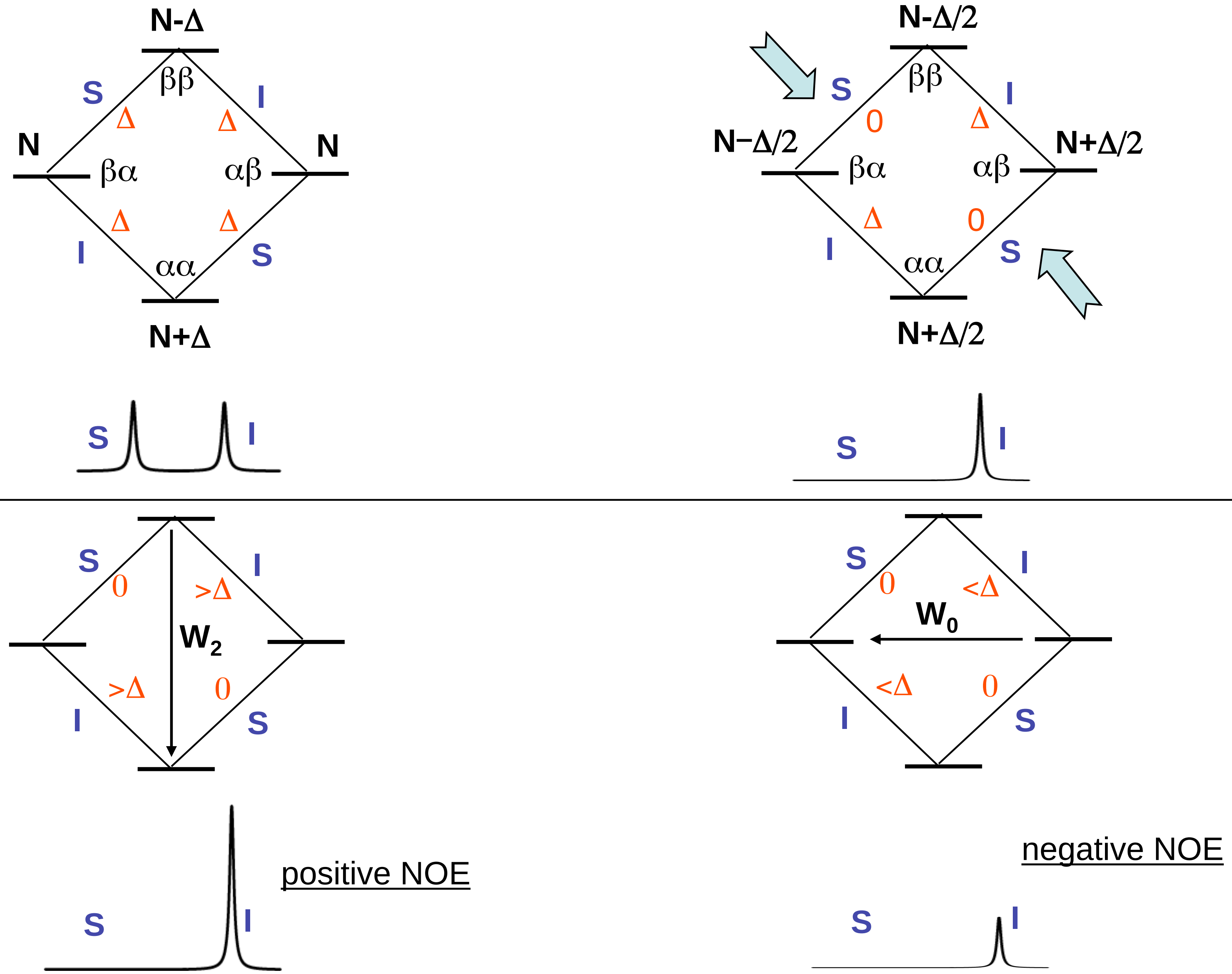


Only single transitions can by observed by NMR (W1)

W_0 and W_2 are cross-relaxation pathways, responsible for the NOE

NMR Spectroscopy

Nuclear Overhauser Effect (NOE)



NMR Spectroscopy

Nuclear Overhauser Effect (NOE)

W_1 tends to reduce the magnitude of the NOE

Saturating for a period of time that is long relative to the relaxation times allows a new **steady-state of populations** to arise

$$\eta_I\{S\} = \left[\frac{W_2 - W_0}{W_0 + 2W_1^I + W_2}\right] \equiv \left[\frac{\sigma_{IS}}{\rho_{IS}}\right]$$

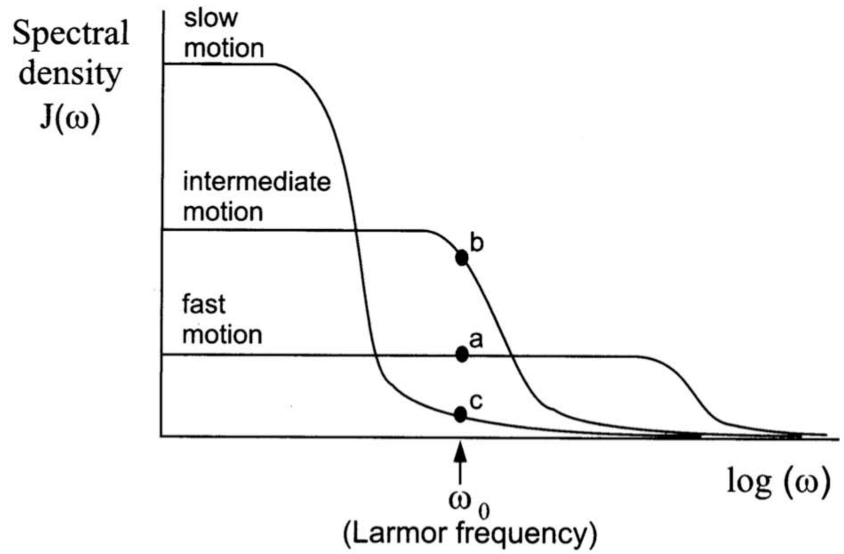
σ_{IS}, cross-relaxation rate: dictates the sign of the NOE

ρ_{IS}, dipolar longitudinal relaxation rate of spin I: it serves to reduce the magnitude

Thus, NOE is related to molecular motion!

NMR Spectroscopy

Nuclear Overhauser Effect (NOE)



^1H at 400 MHz

W_1 at 400 MHz

W_2 at 800 MHz (**W_I+W_S**)- stimulated by rapidly tumbling molecules

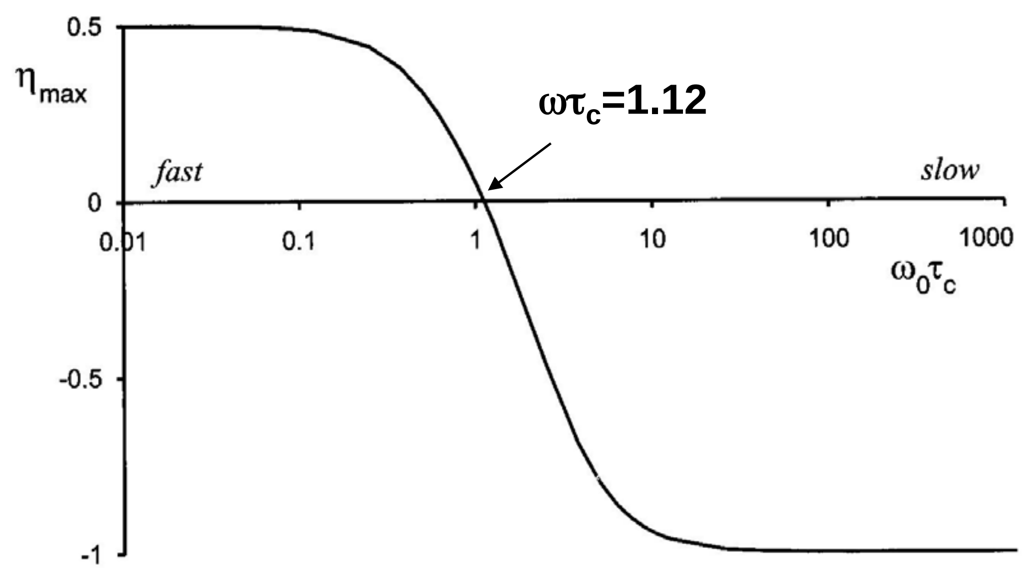
W_0 at Hz-kHZ (|**W_I-W_S**|)- stimulated by slowly tumbling molecules

Small molecules exhibit **positive** NOEs

Large molecules exhibit **negative** NOEs

NMR Spectroscopy

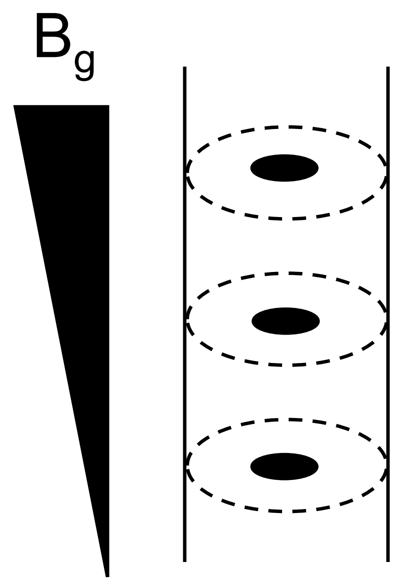
Nuclear Overhauser Effect (NOE)



Variation in NOE as a function of molecular tumbling rates

NMR Spectroscopy

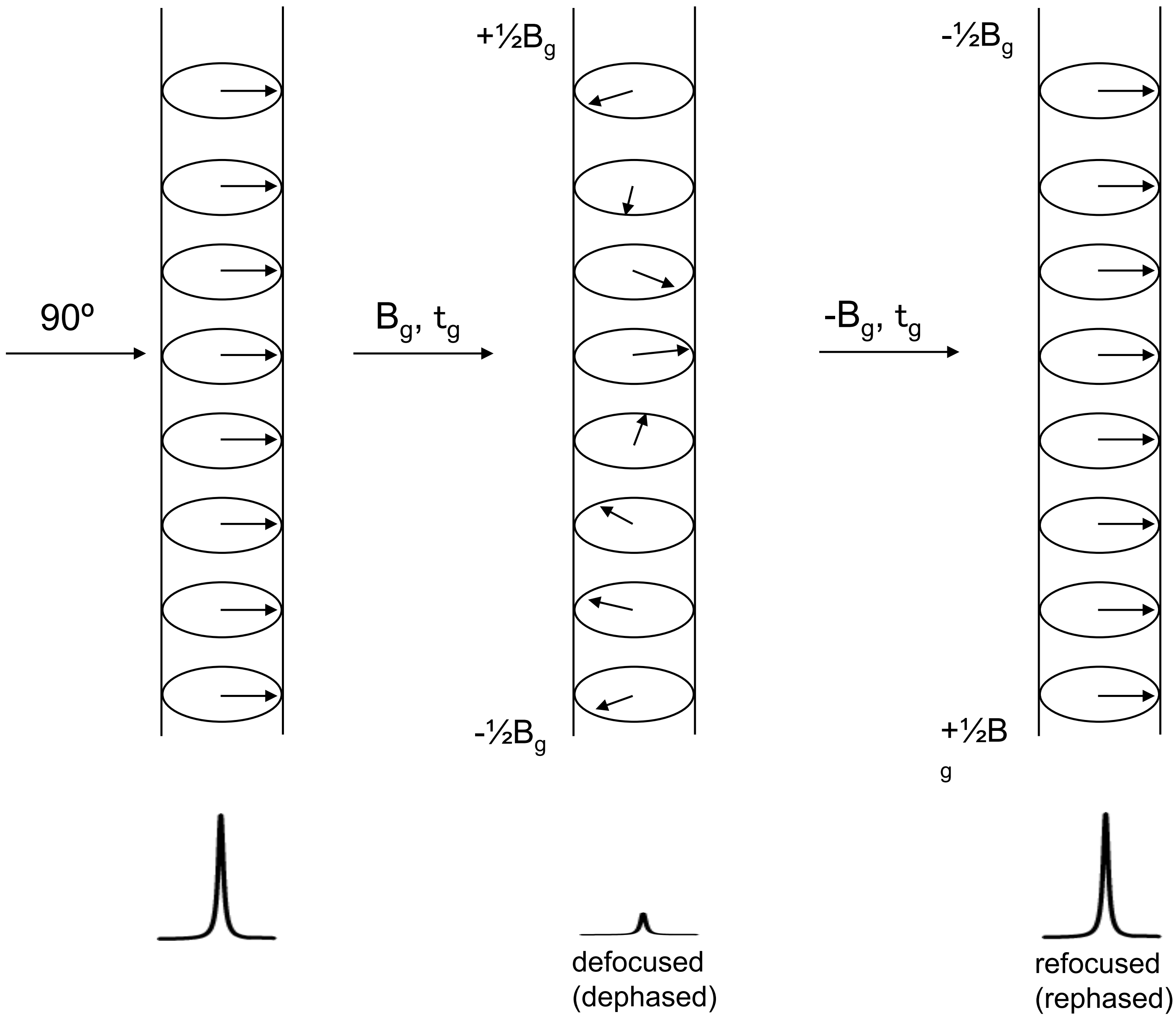
Field gradient



Variation of magnetic field strength along the z axis

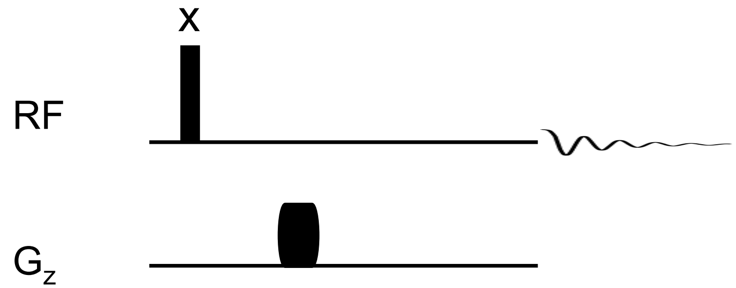
NMR Spectroscopy

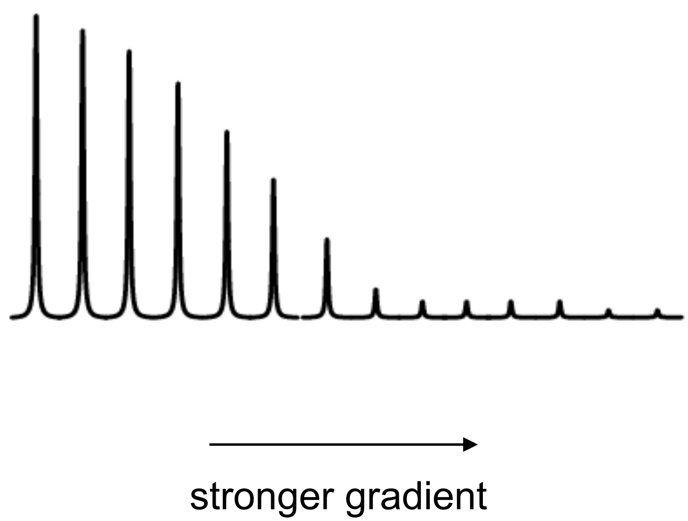
Field gradient



NMR Spectroscopy

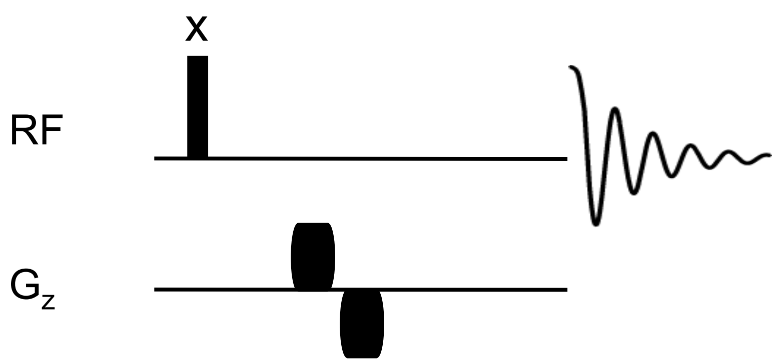
Field gradient

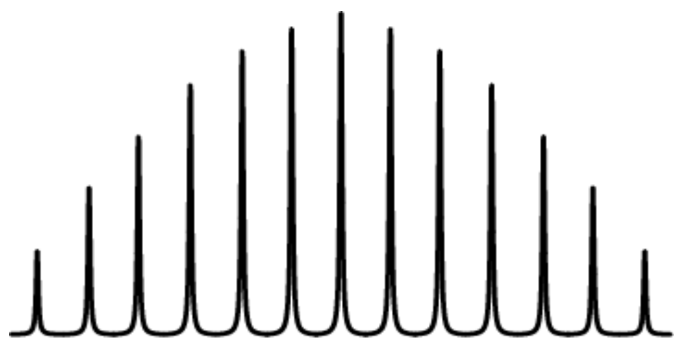




NMR Spectroscopy

Field gradient

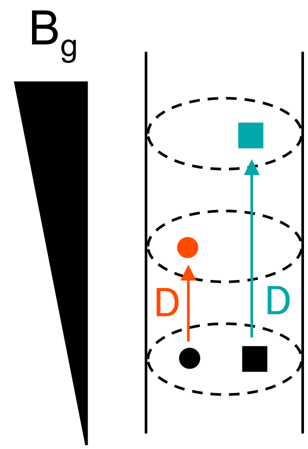


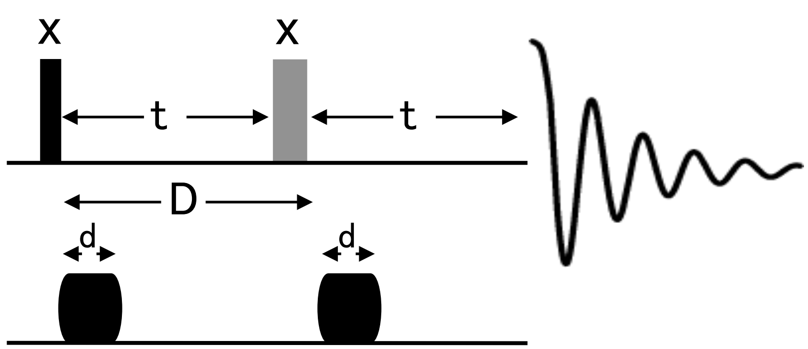


Variation of the second gradient pulse (90 to 110% of the first)

NMR Spectroscopy

Diffusion-ordered spectroscopy





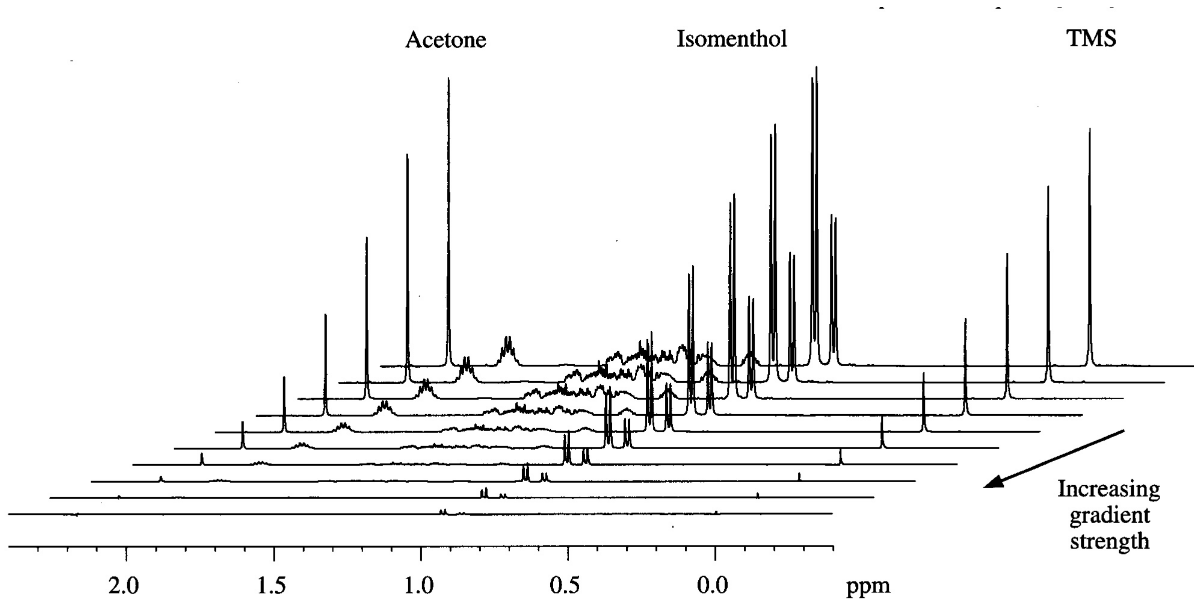
$$I = I_0 \exp\left(\frac{-2\tau}{T_2} - (\gamma \delta G)^2 D \left(\Delta - \frac{\delta}{3} \right) \right)$$

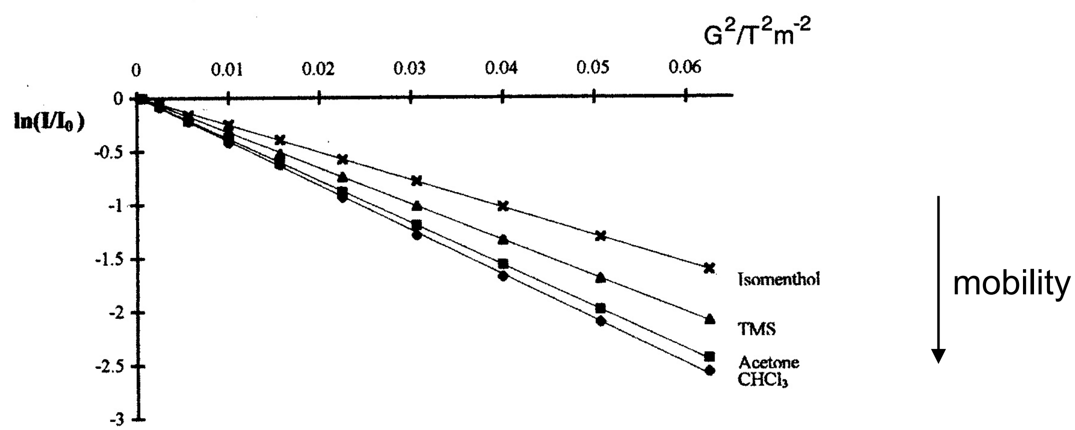
G gradient strength

D diffusion coefficient

NMR Spectroscopy

Diffusion-ordered spectroscopy

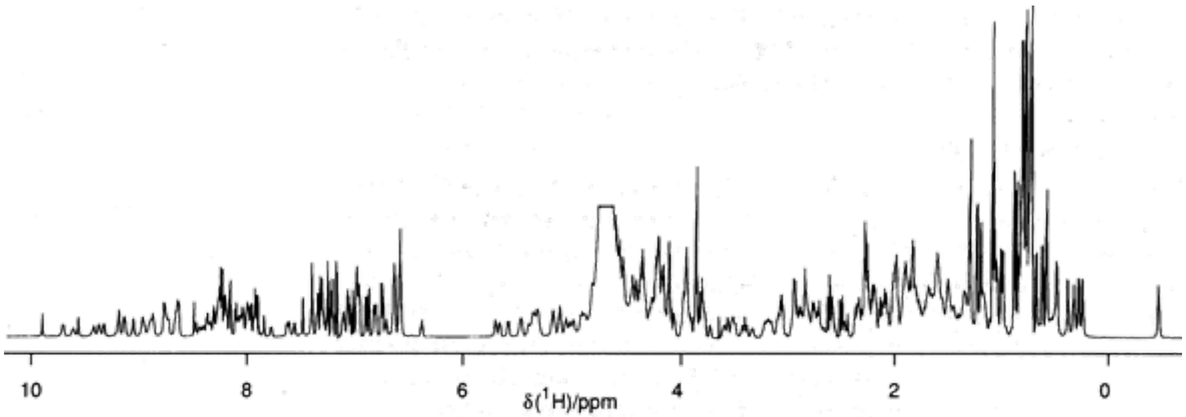




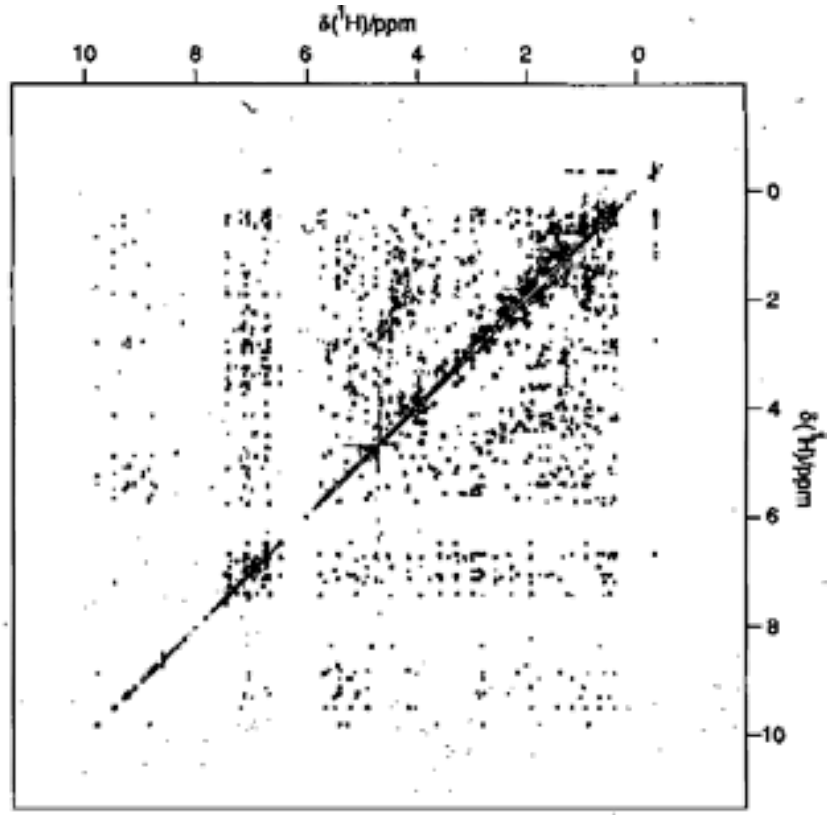
NMR Spectroscopy

Multi-dimensional NMR

One dimension



Two dimensions



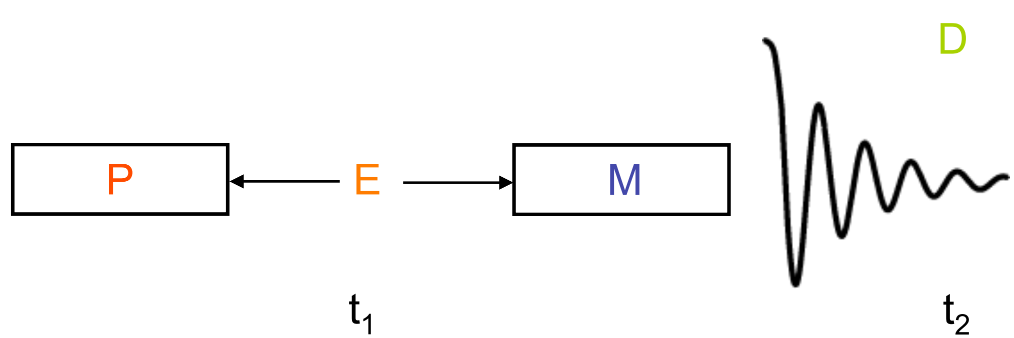
NMR Spectroscopy

Multi-dimensional NMR

To generate a spectrum with two frequency domains, f_1 and f_2, it is necessary to sample data as a function of two separate time variables, t_1 and t_2.

General scheme for 2D NMR experiment

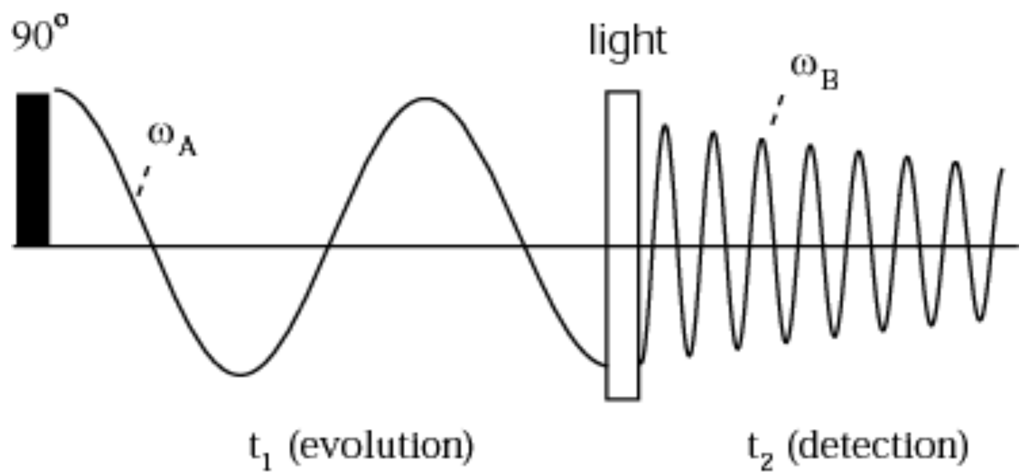
P: Preparation
E: Evolution
M: Mixing
D: Detection



NMR Spectroscopy

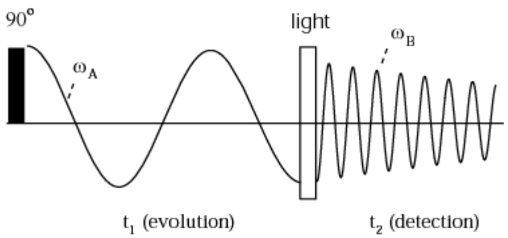
Multi-dimensional NMR

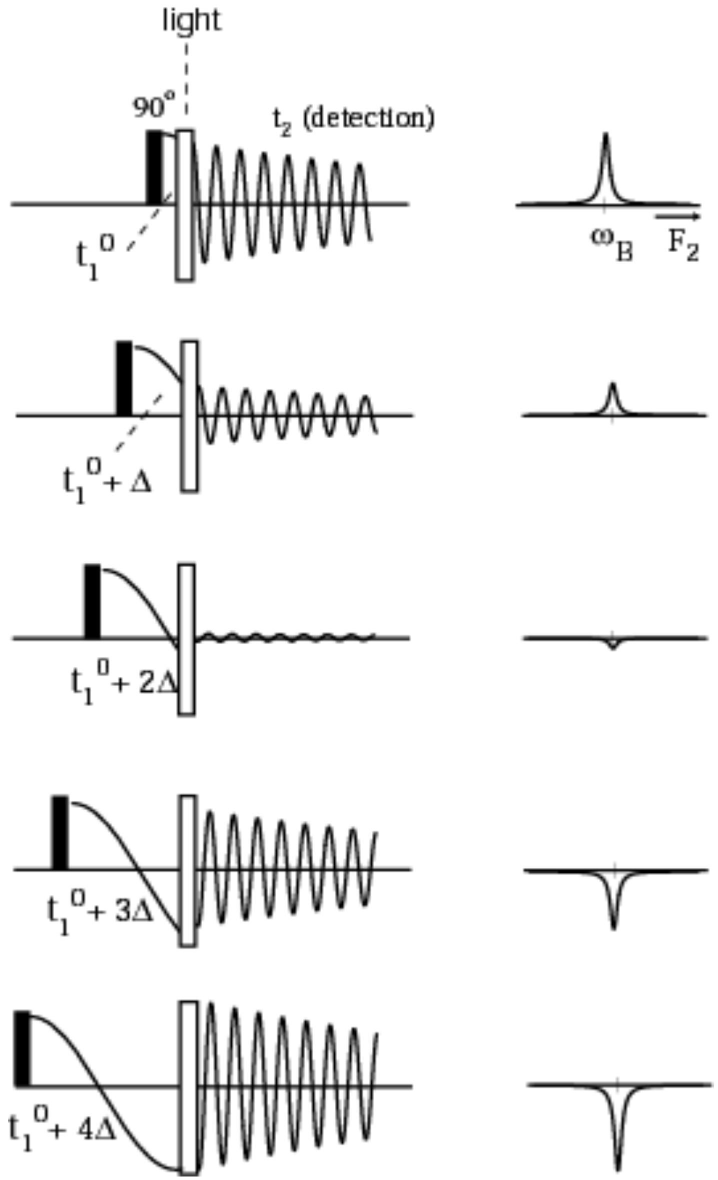
$$A \xrightarrow{h\nu} B$$



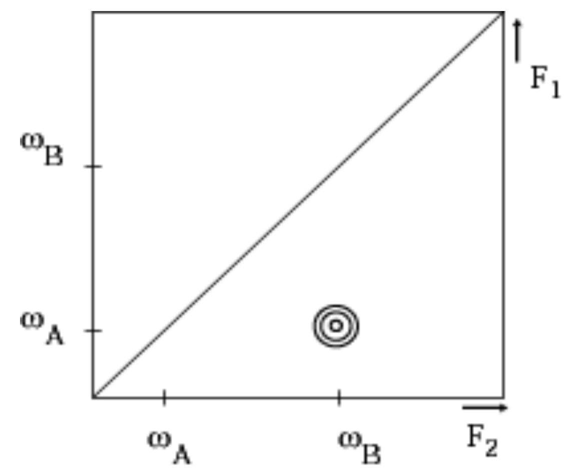
NMR Spectroscopy

Multi-dimensional NMR





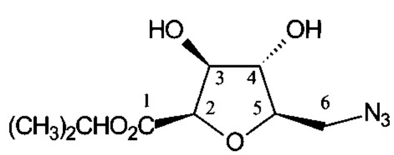
$$S(t_1,t_2) \xrightarrow[t_2 \curvearrowright F_2]{FT} S(t_1,F_2) \xrightarrow[t_1 \curvearrowright F_1]{FT} S(F_1,F_2)$$

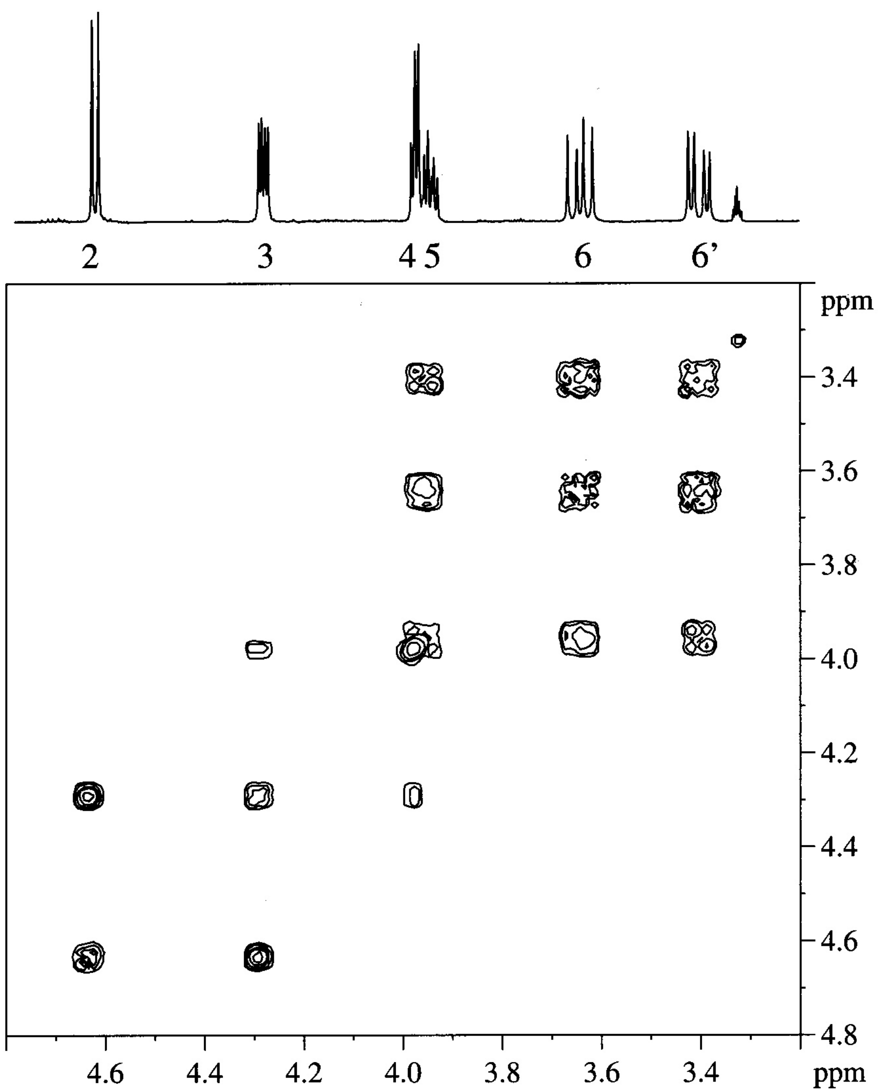


NMR Spectroscopy

COSY (COrrelated SpectroscopY)

Correlation through bonds (J-coupling)

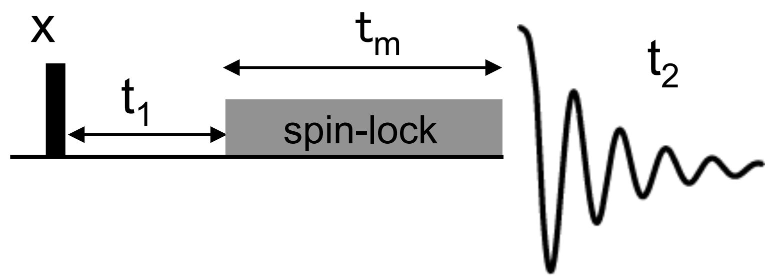


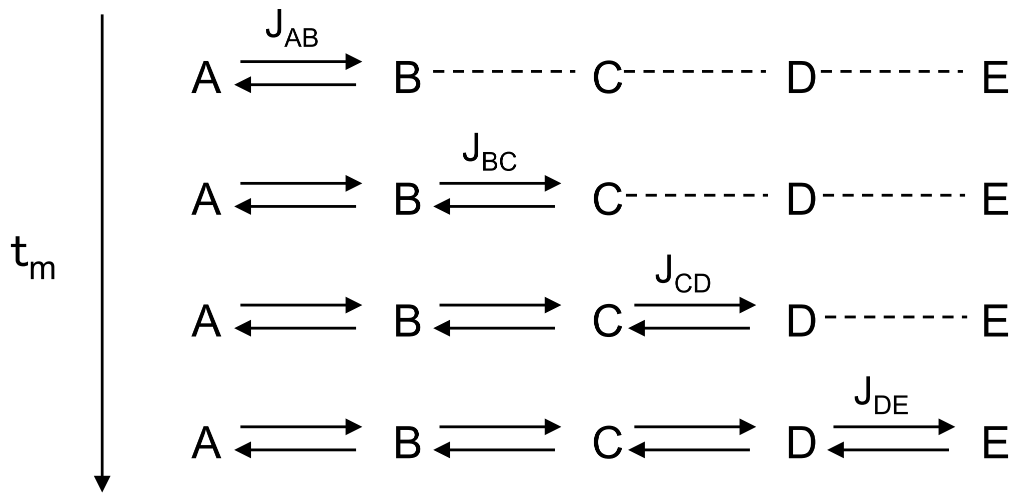


NMR Spectroscopy

TOCSY (Total COrrelated SpectroscopY)

Correlation through bonds (J-coupling)

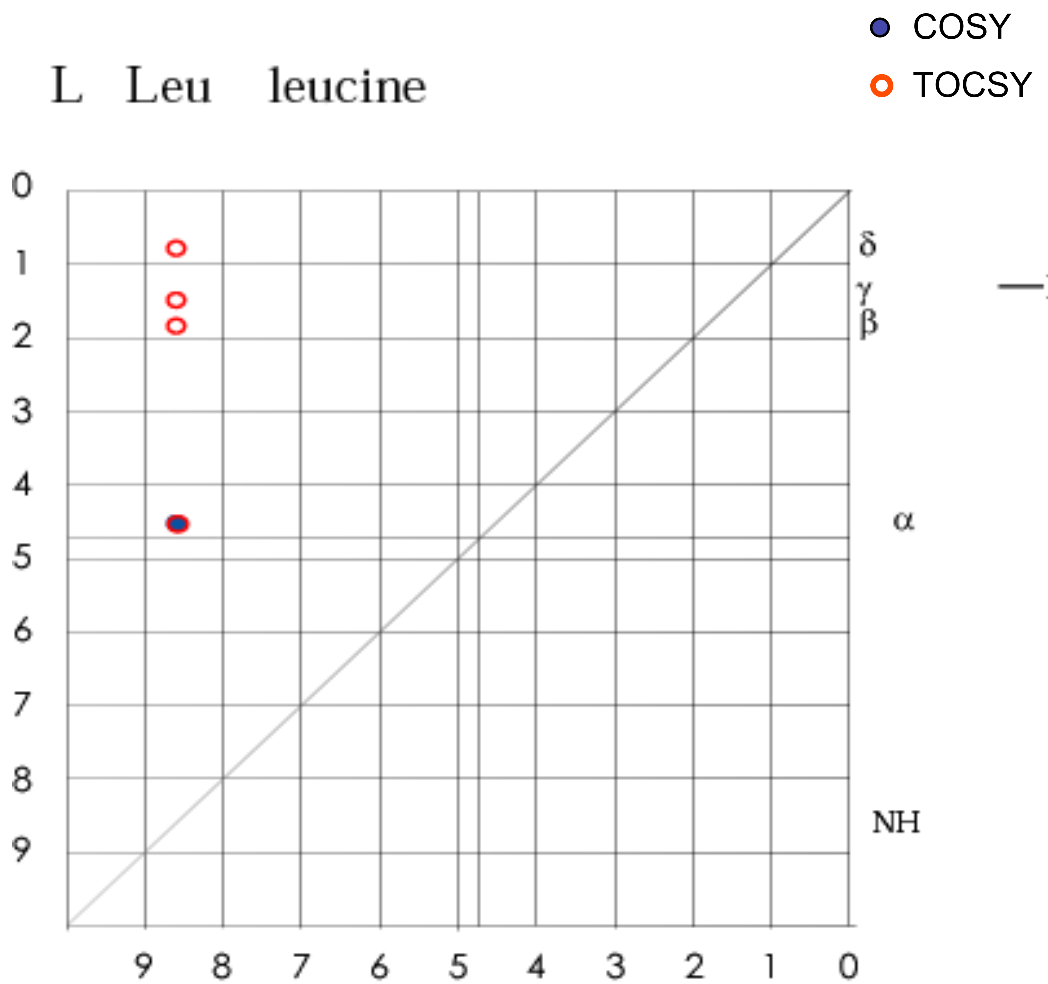


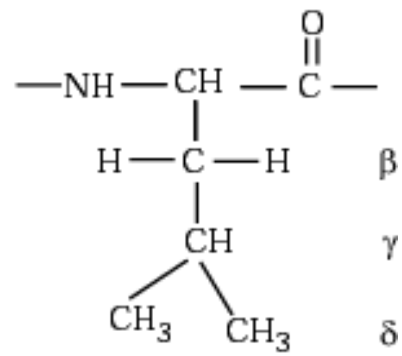


NMR Spectroscopy

COSY vs. TOCSY

Correlation through bonds (J-coupling)

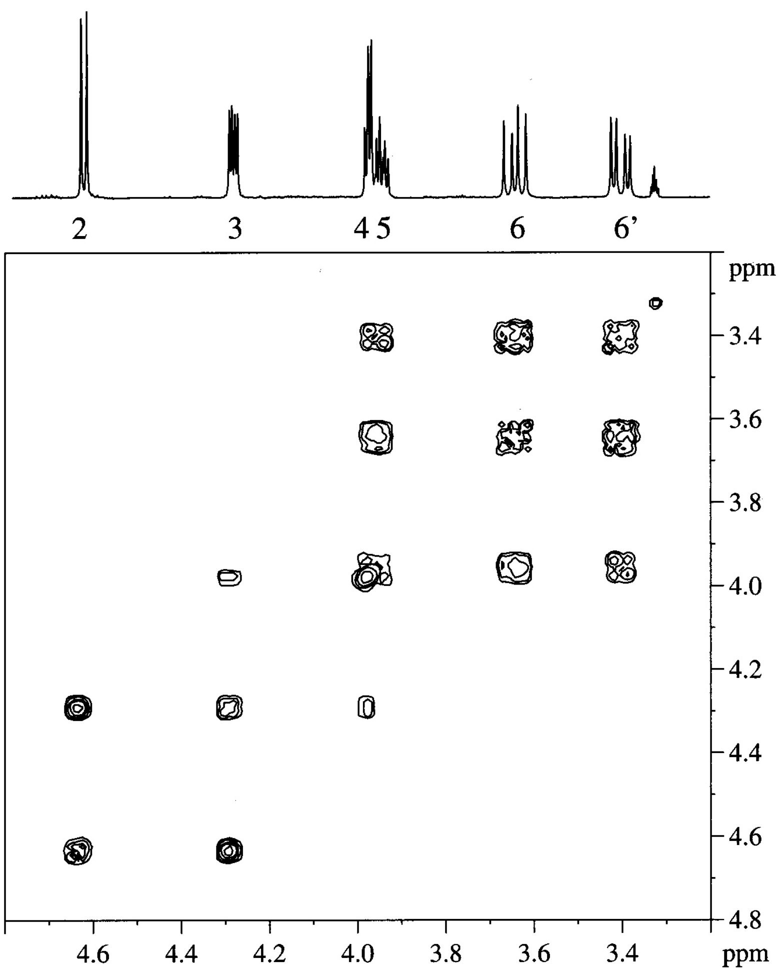


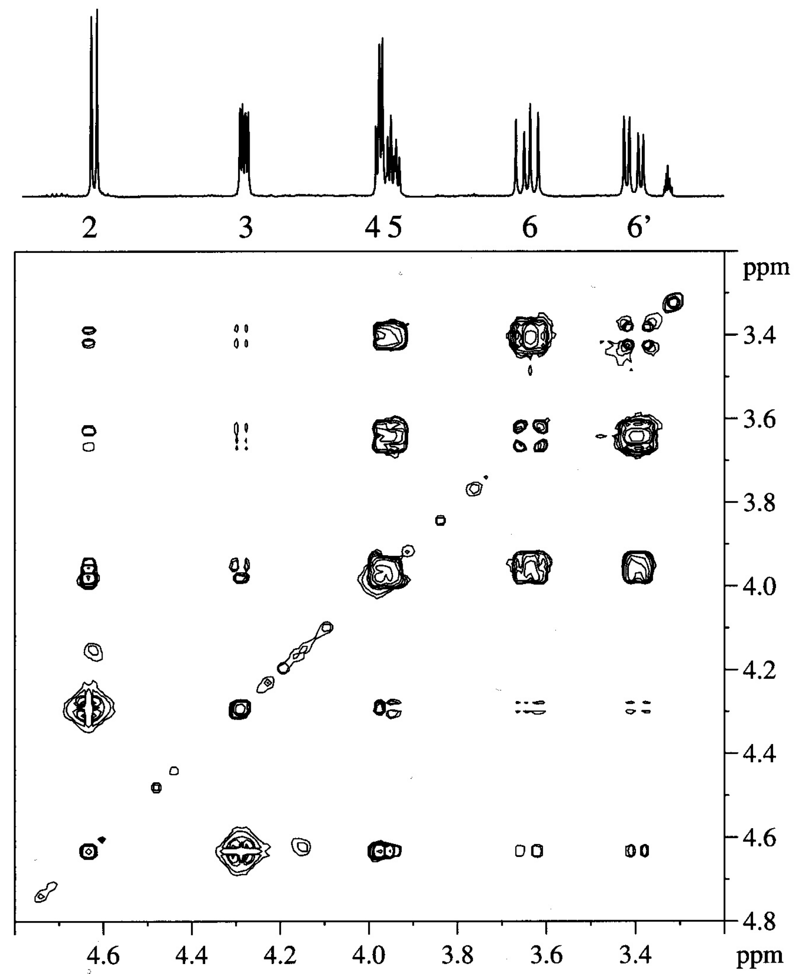


NMR Spectroscopy

COSY vs. TOCSY

Correlation through bonds (J-coupling)

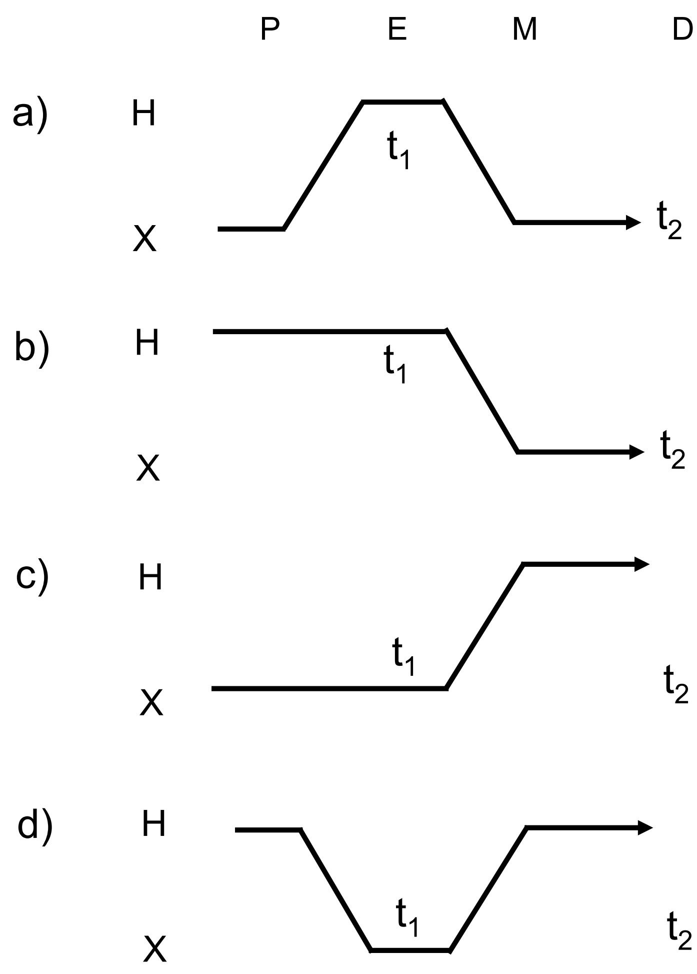




NMR Spectroscopy

General schemes for 2D NMR

$$\frac{S}{N} \propto T^{-1} B_0^{3/2} \gamma_{exc} \gamma_{obs}^{3/2} T_2^* (NS)^{1/2}$$

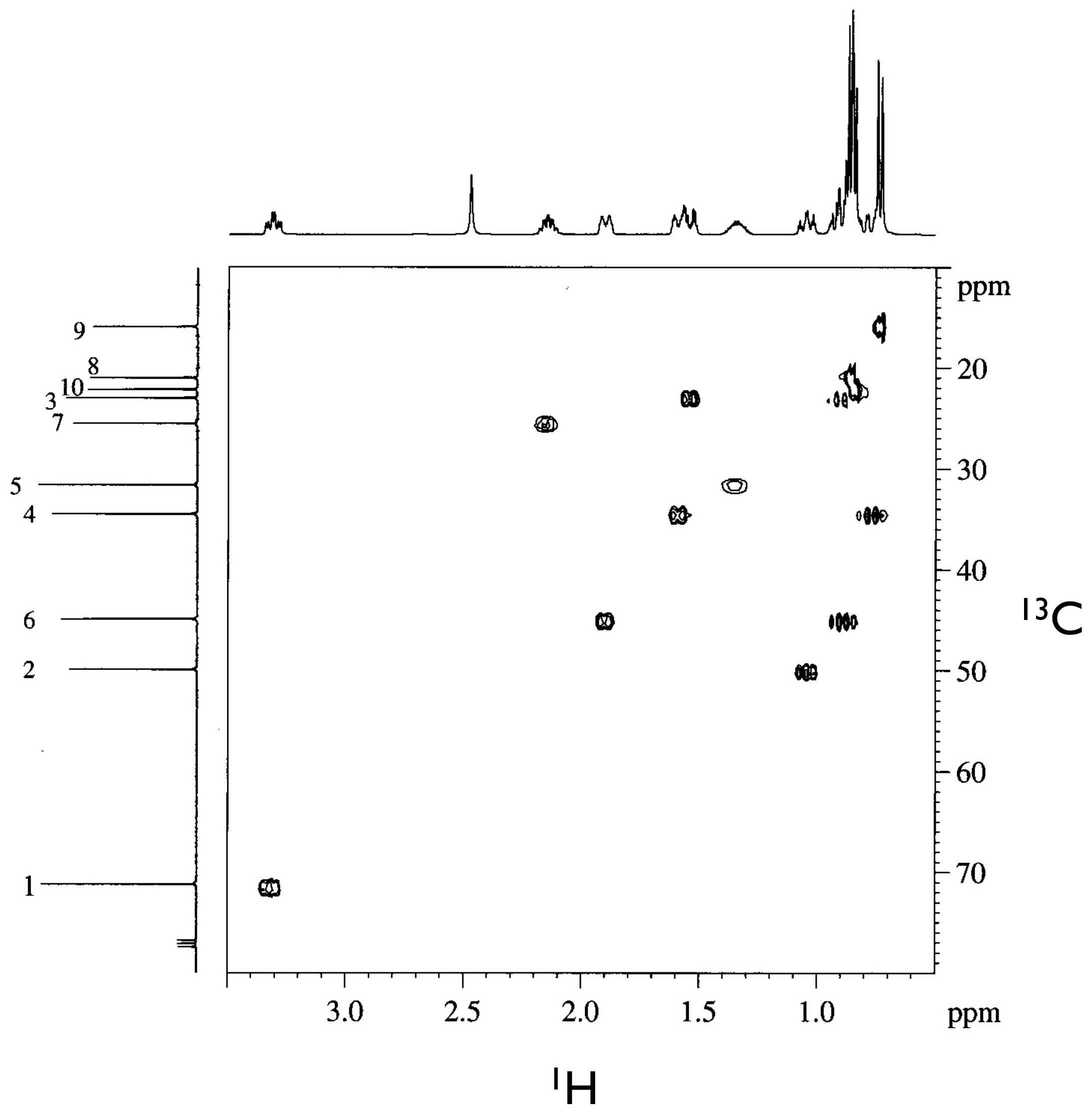


Relative sensitivity

	^1H-^{31}P	^1H-^{13}C	^1H-^{15}N
a)	1	1	1
b) (traditional)	2.5	4	10
c)	4	8	30
d) (inverse) modern	10	32	300

NMR Spectroscopy

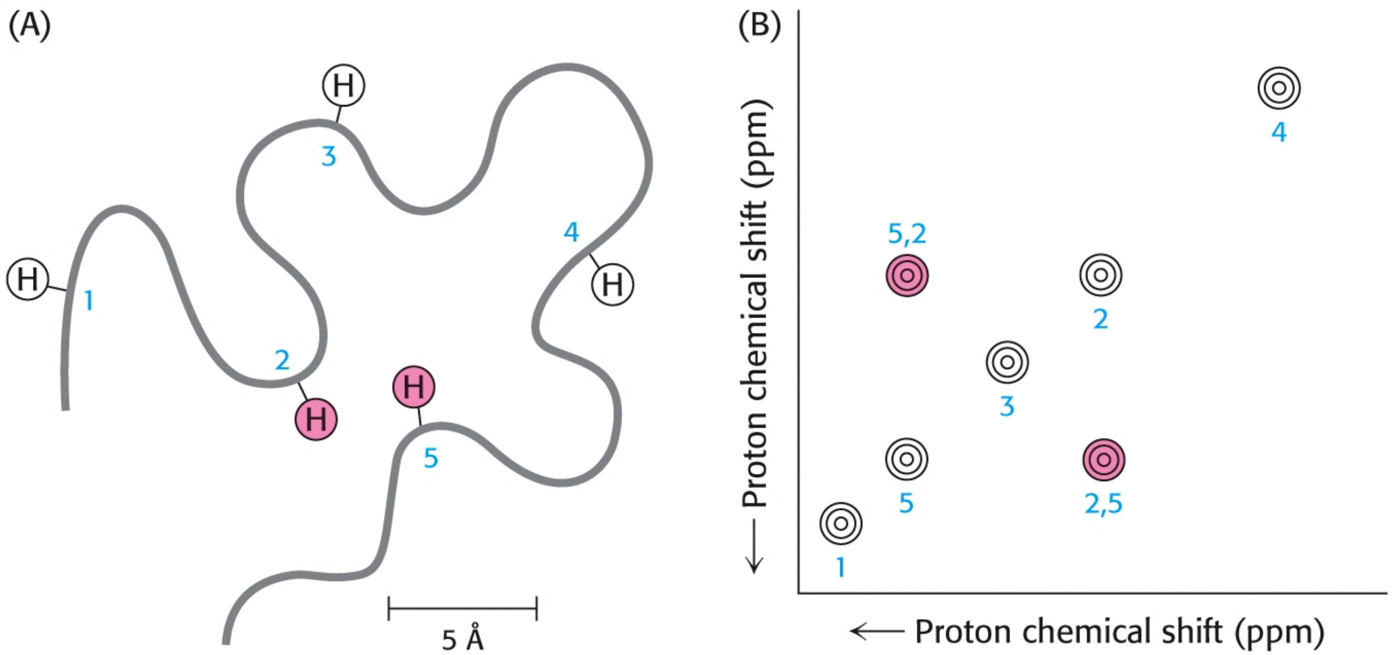
Heteronuclear Single Quantum Coherence (HSQC)



NMR Spectroscopy

Protein NMR

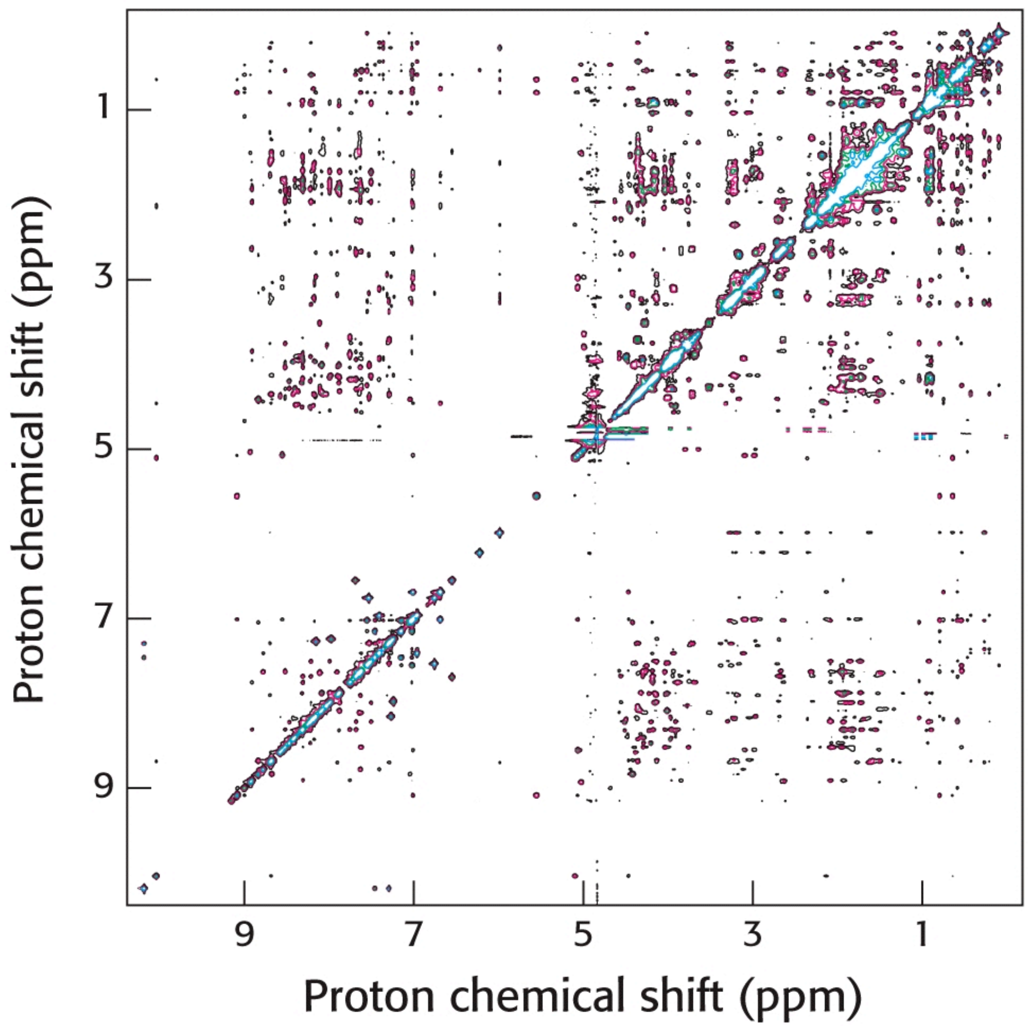
2D NOESY



NMR Spectroscopy

Protein NMR

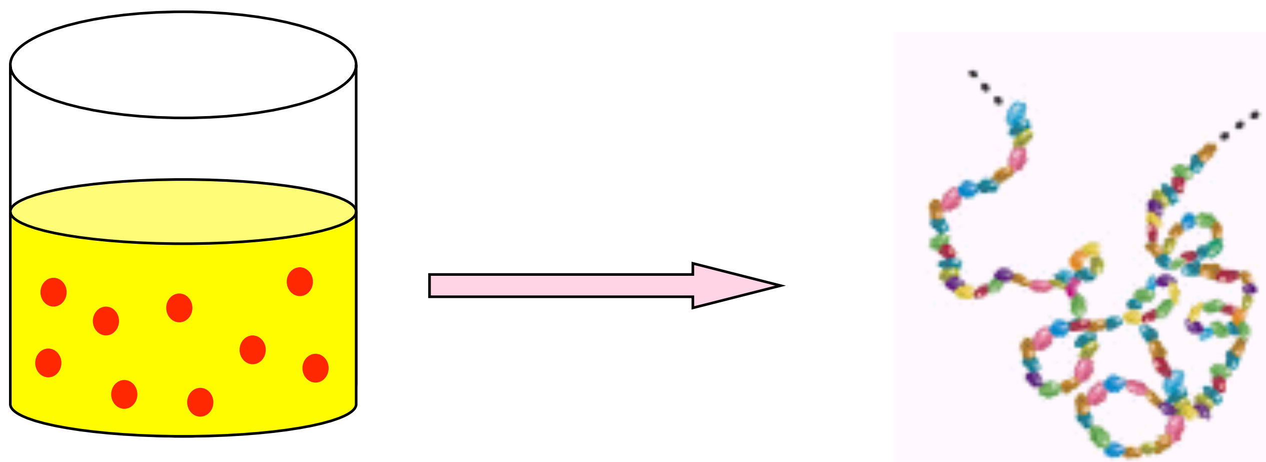
2D NOESY



NMR Spectroscopy

Protein NMR

Isotopically labeled proteins



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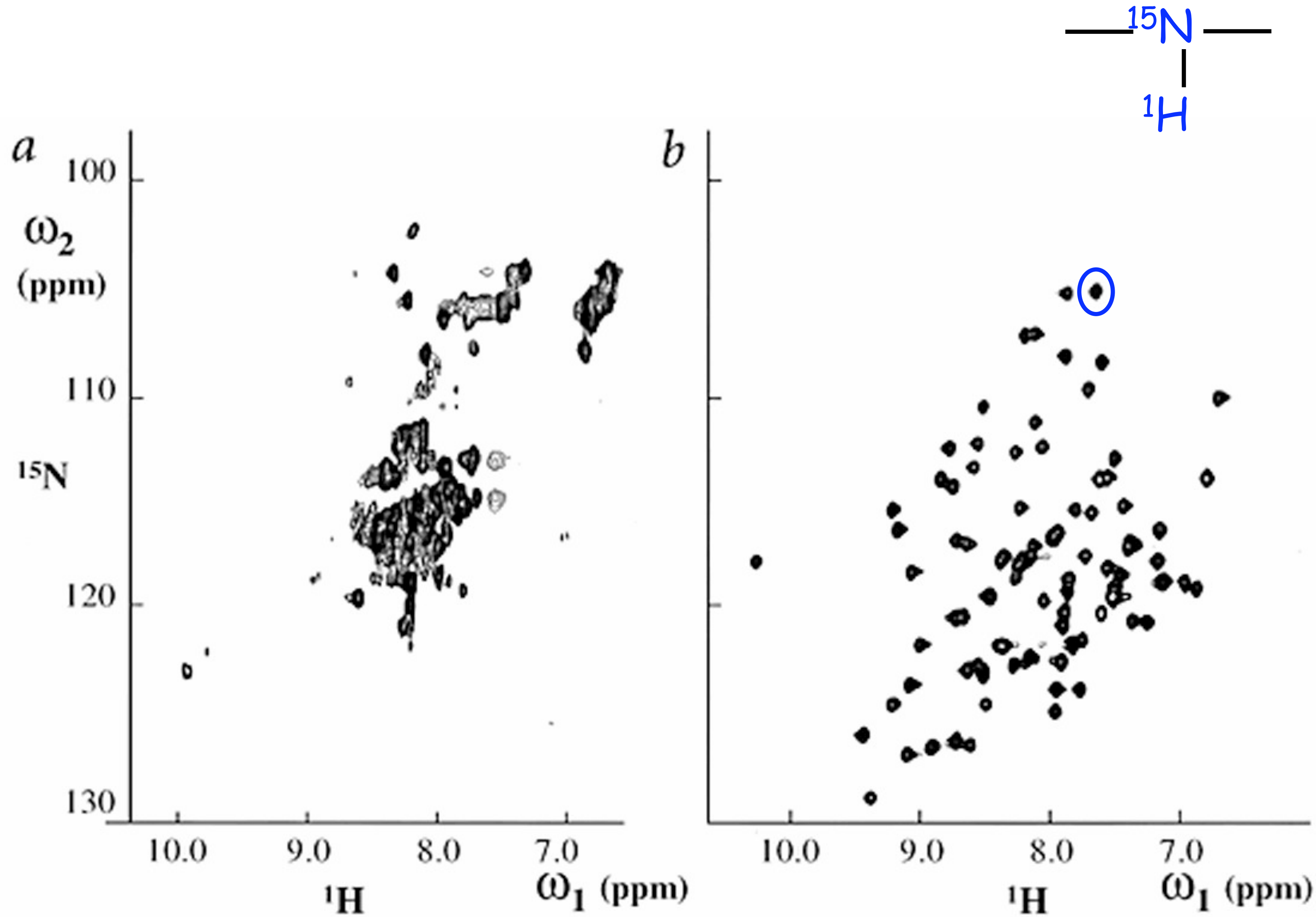
NMR Spectroscopy

Protein NMR

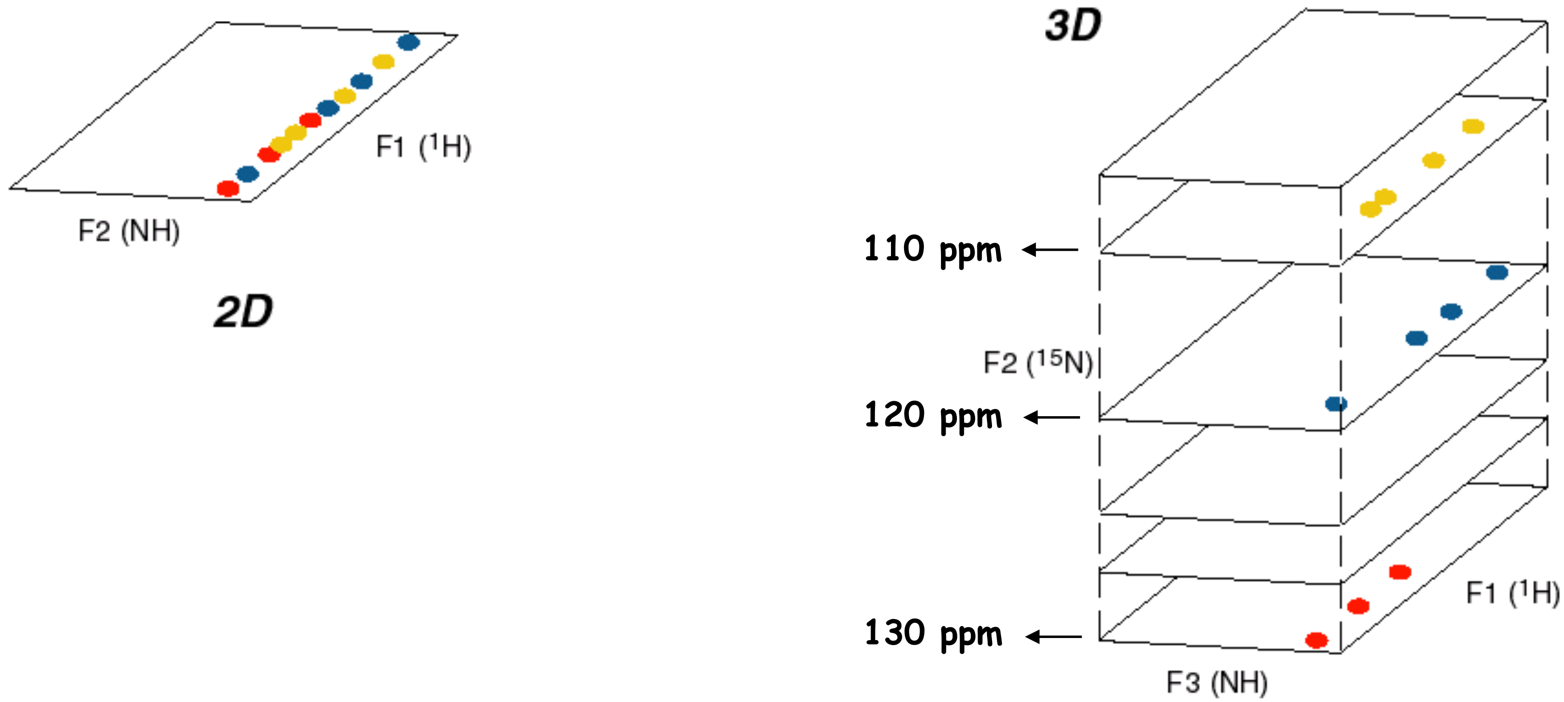
^1H-^{15}N HSQC (protein's fingerprint)

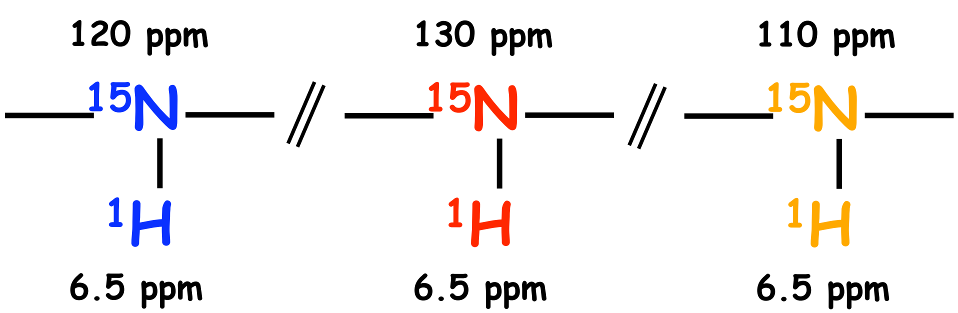


NMR Spectroscopy

Protein NMR

Signal overlap problem alleviated by 3D & 4D NMR

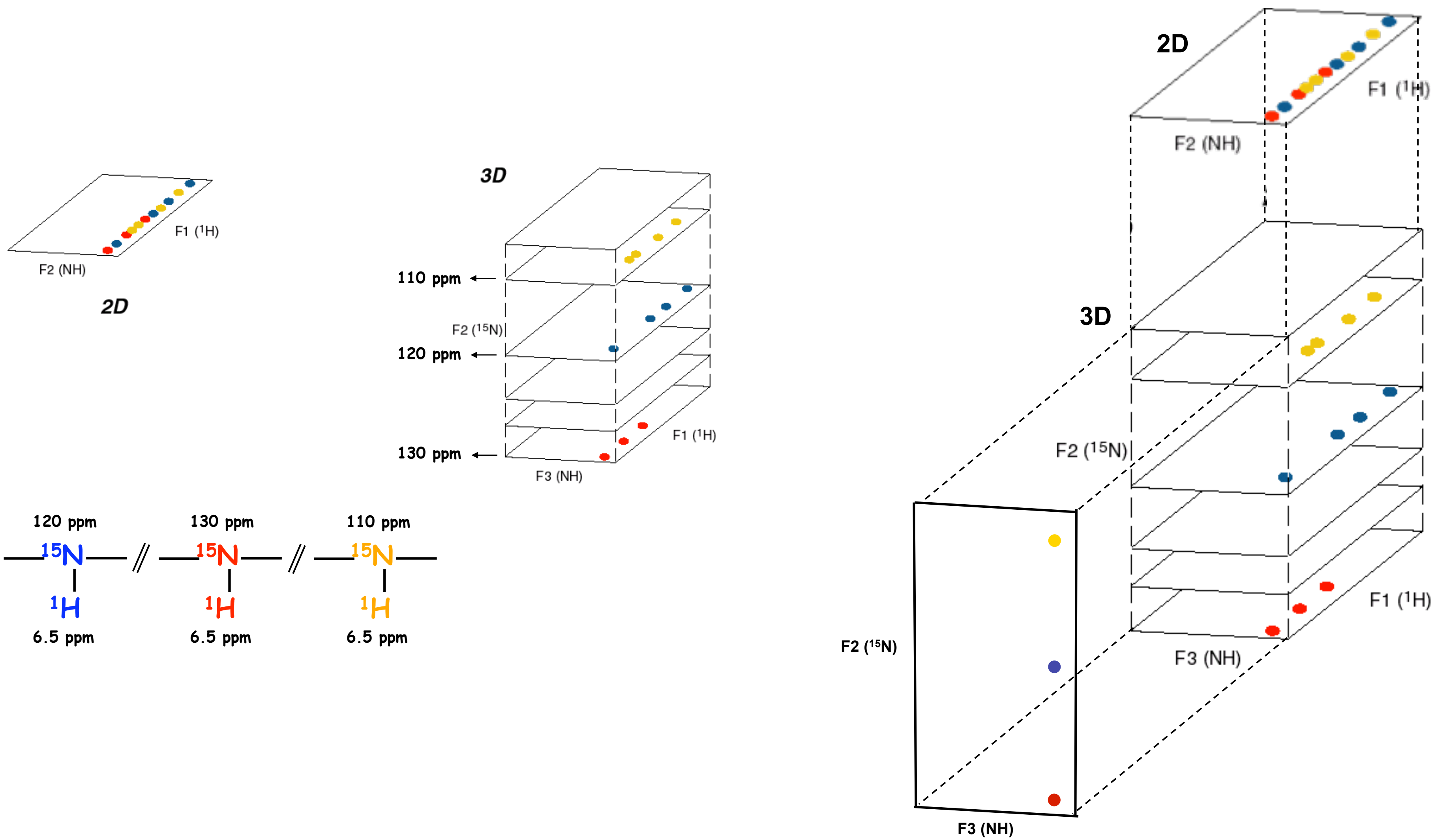




NMR Spectroscopy

Protein NMR

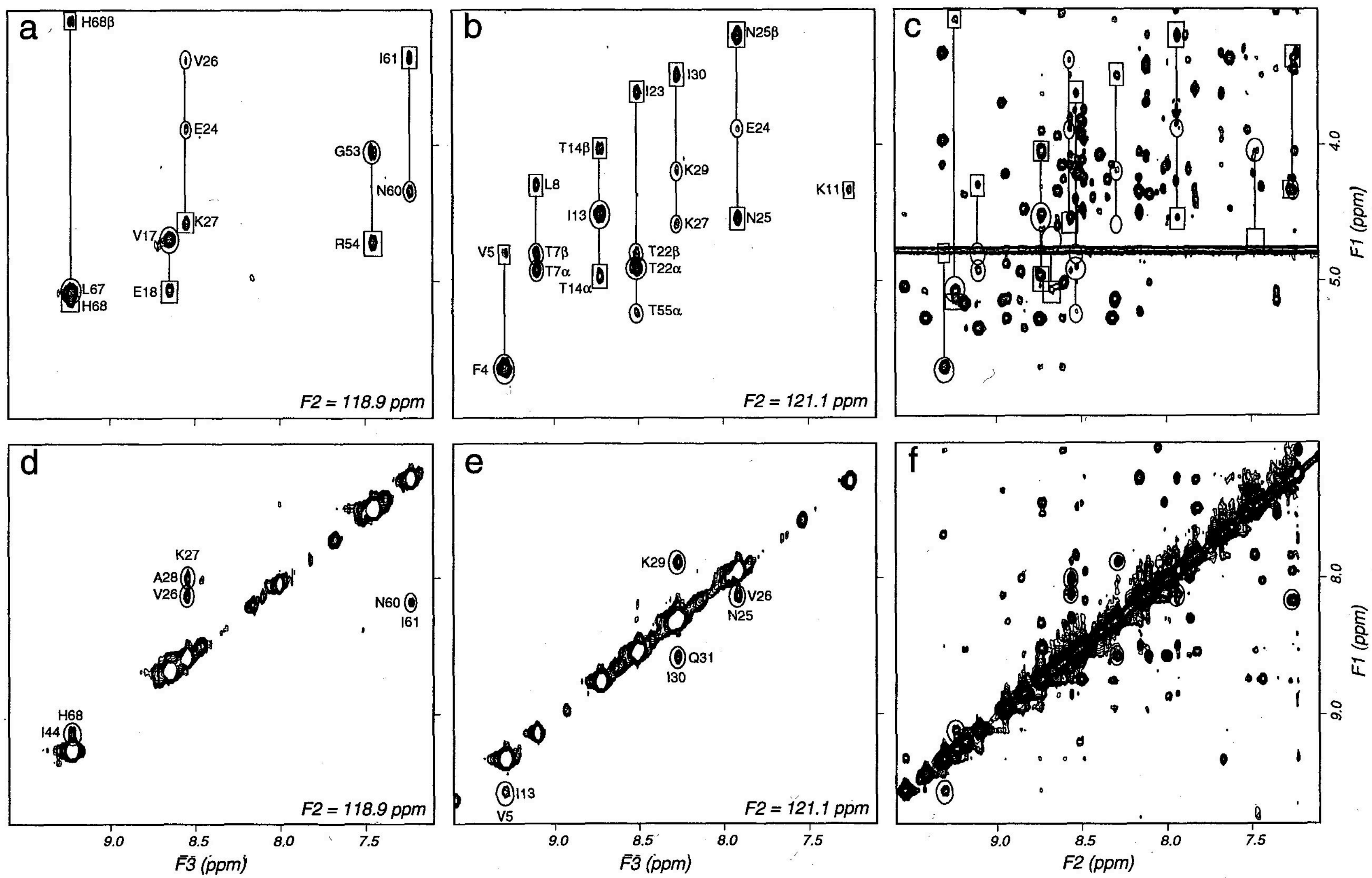
Signal overlap problem alleviated by 3D & 4D NMR



NMR Spectroscopy

Protein NMR

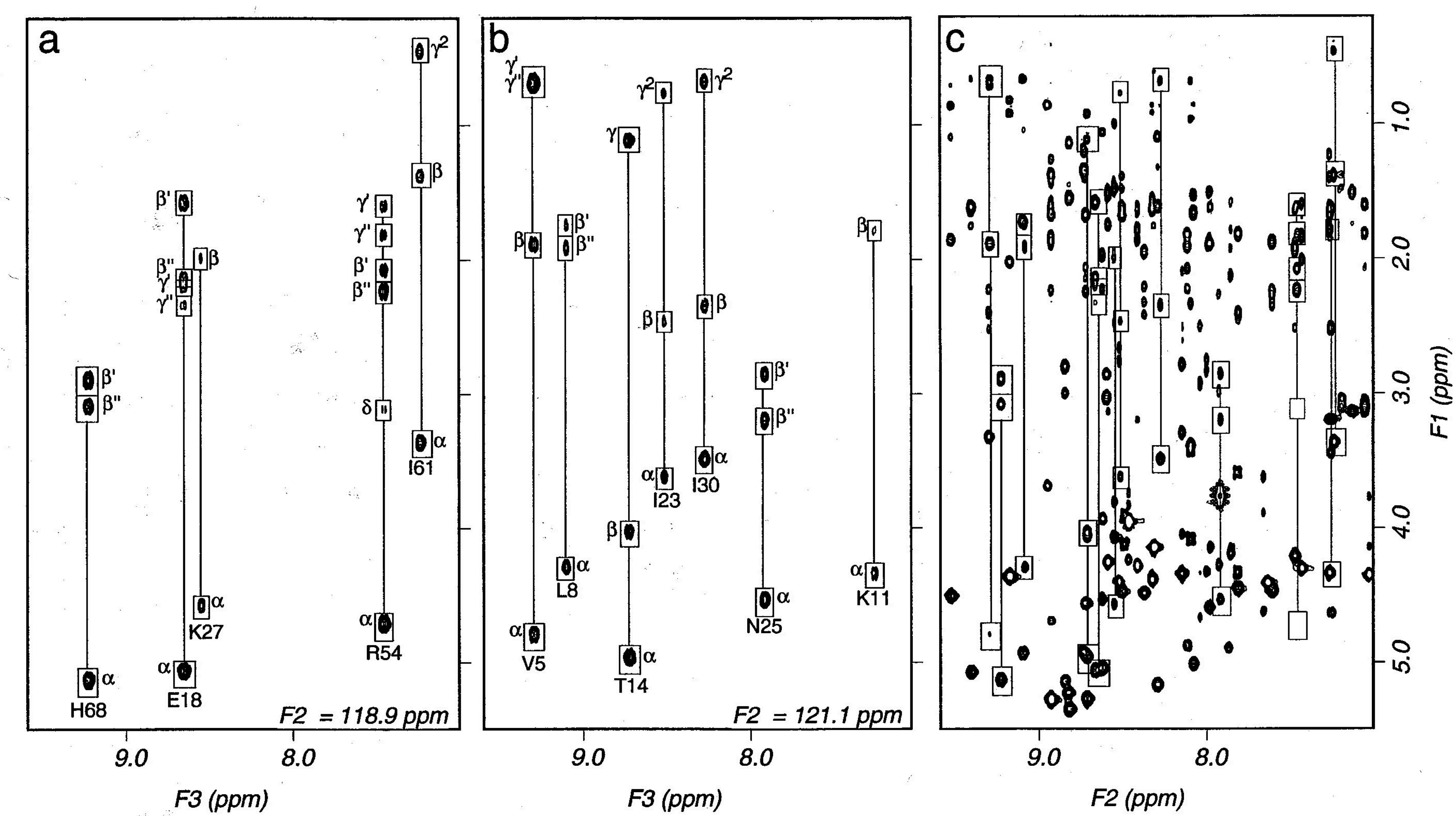
Signal overlap problem alleviated by 3D & 4D NMR



NMR Spectroscopy

Protein NMR

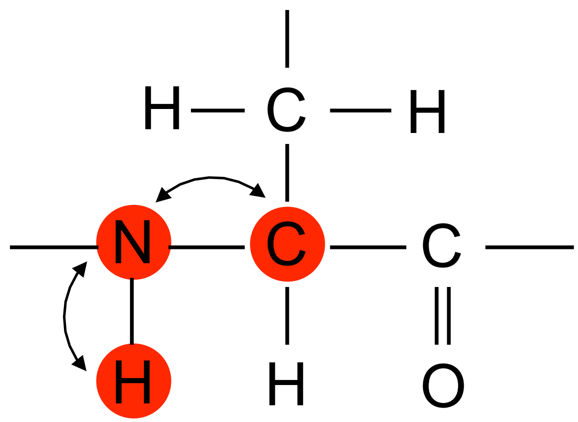
Signal overlap problem alleviated by 3D & 4D NMR



NMR Spectroscopy

Protein NMR

Assignment - Triple Resonance Experiments

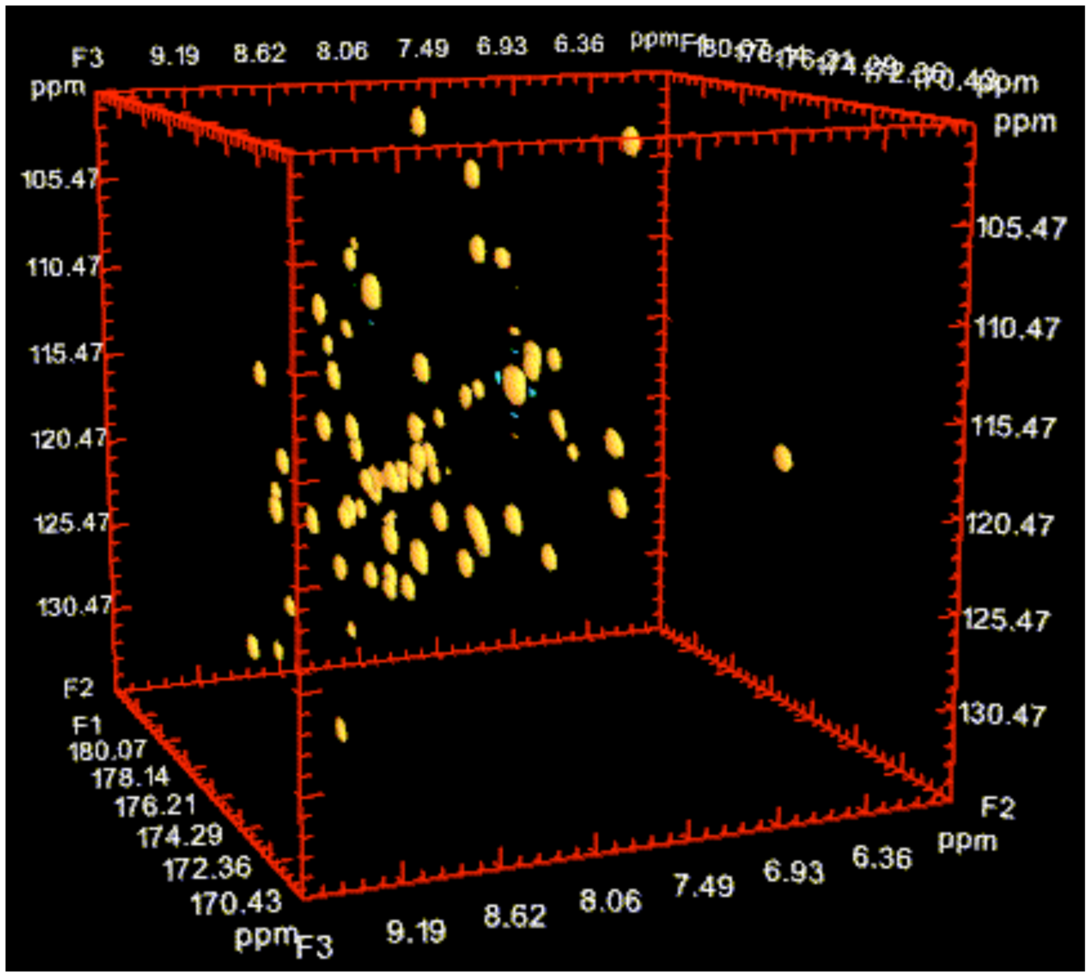


3D HNCA

NMR Spectroscopy

Protein NMR

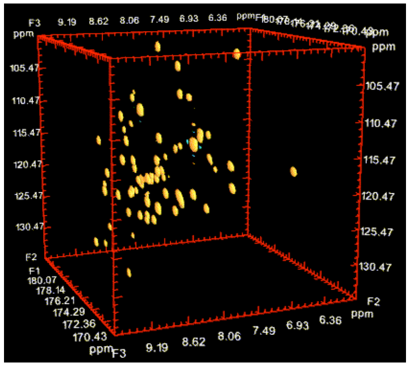
Assignment - Triple Resonance Experiments

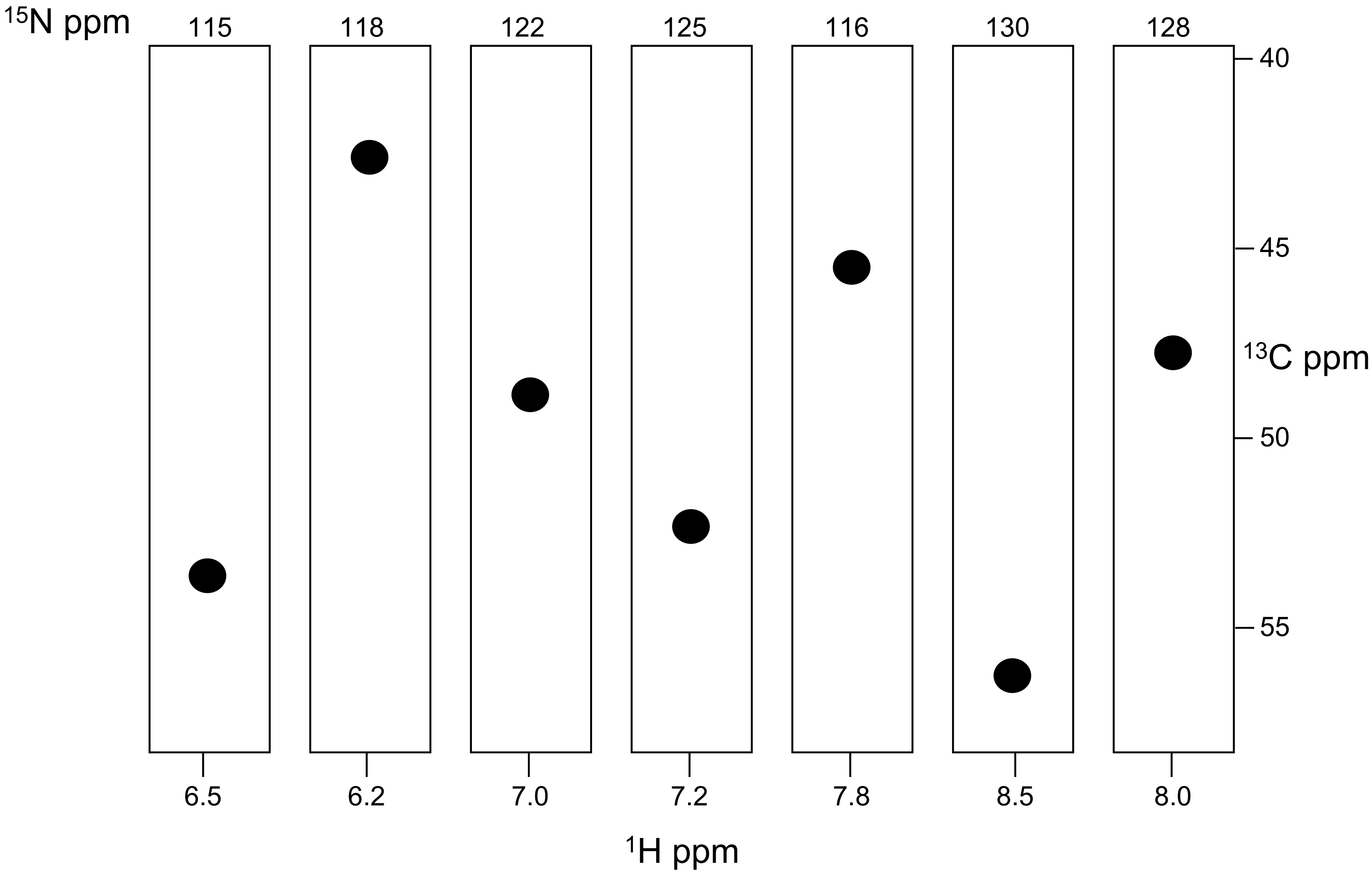


NMR Spectroscopy

Protein NMR

Assignment - Triple Resonance Experiments

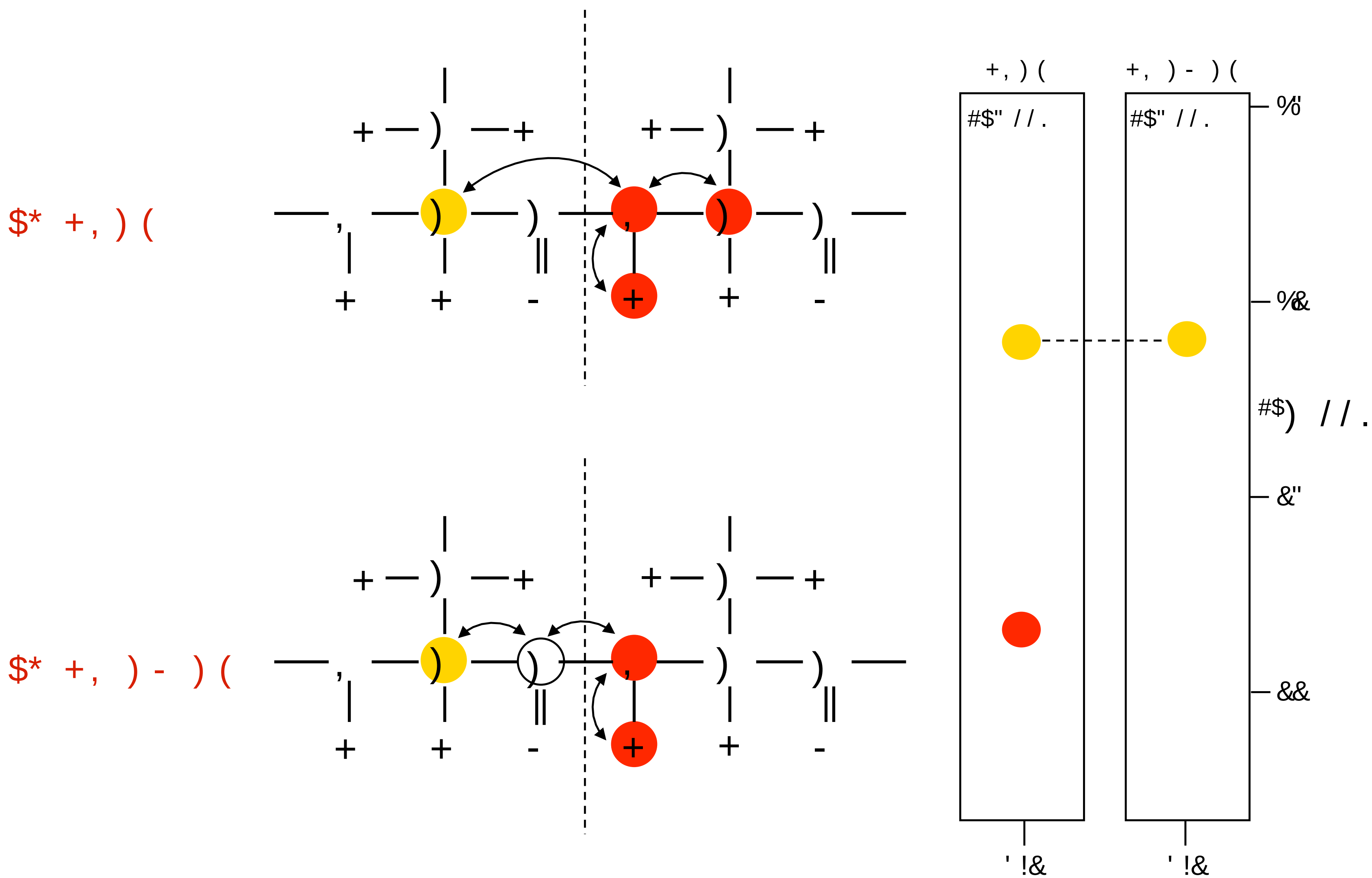




NMR Spectroscopy

Protein NMR

Assignment - Triple Resonance Experiments



NMR Spectroscopy

Protein NMR

Assignment - Triple Resonance Experiments