Two Dimensional NMR-Part I

DR. DARSHANA MEHTA

SCHOOL OF STUDIES IN CHEMISTRY AND BIOCHEMISTRY, VIKRAM UNIVERSITY, UJJAIN – 456 010, MADHYA PRADESH, INDIA
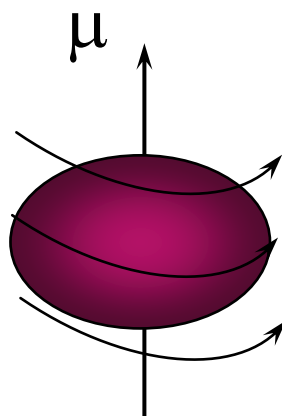
Nuclear Magnetic Resonance

Nuclear spin

$\mu = \gamma I h$

μ - magnetic moment

γ - gyromagnetic ratio
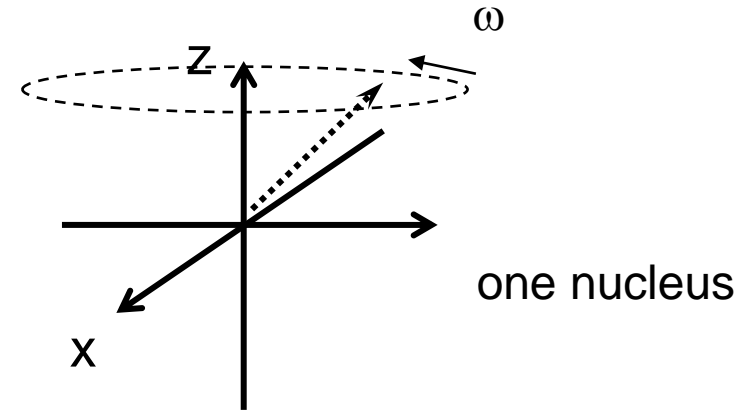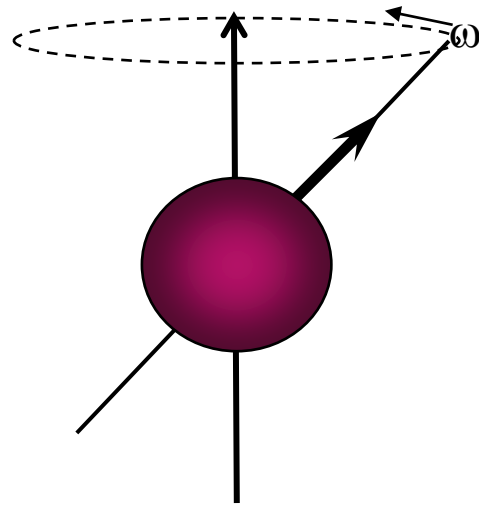
I - spin quantum number

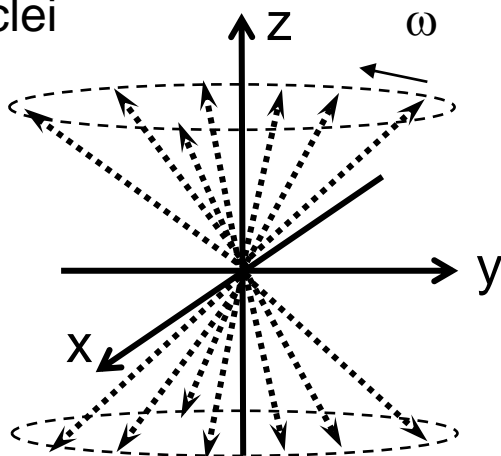h - Planck's constant

I is a property of the nucleus

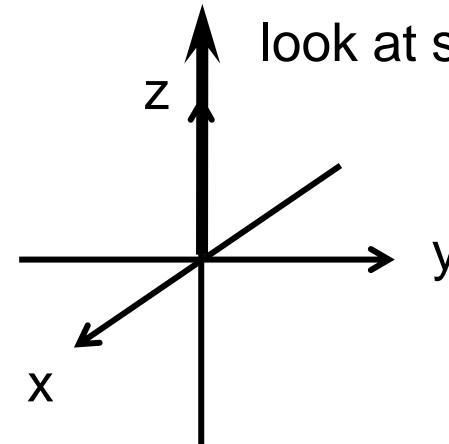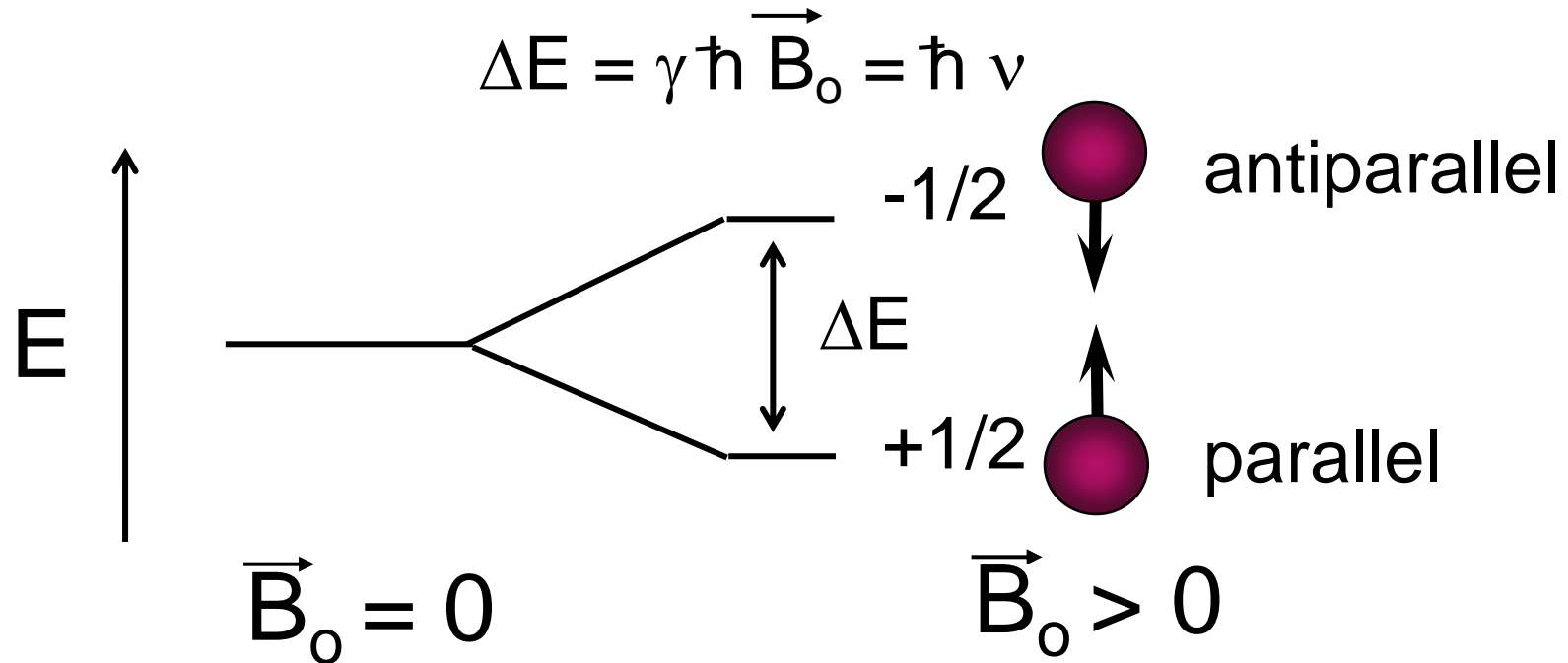| Mass No.	Atomic No.	I
Odd	Even or odd	1/2, 3/2, 5/2,...
Even	Even	0
Even	Odd	1, 2, 3

The net magnetization vector

one nucleus

many nuclei

M_o - net magnetization
   vector allows us to
   look at system as a whole

Allowed Energy States for a
Spin 1/2 System

$$\Delta E = \gamma \hbar \vec{B}_o = \hbar \nu$$

antiparallel

-1/2

ΔE

E

+1/2   parallel

$\vec{B}_o = 0$ $\vec{B}_o > 0$

➤Therefore, the nuclei will absorb light with energy E resulting in a change
   of the spin states.

➤Small population difference between +1/2 and -1/2 state

➤It is the small excess of nuclei in the lower state that produce NMR signal

Radio Wave hν

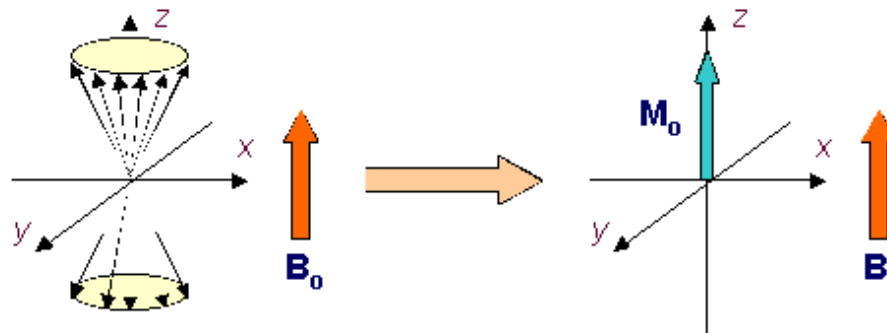**Classical view**

B$_o$

Larmor Equation:

$$\nu \ = \ \gamma \, B_o/ \, 2\pi$$
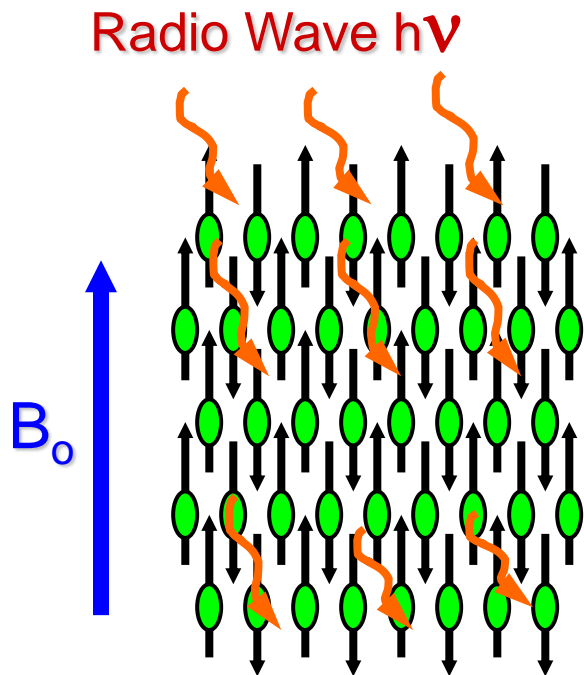
ν = Larmor frequency;
γ = nuclear gyric ratio
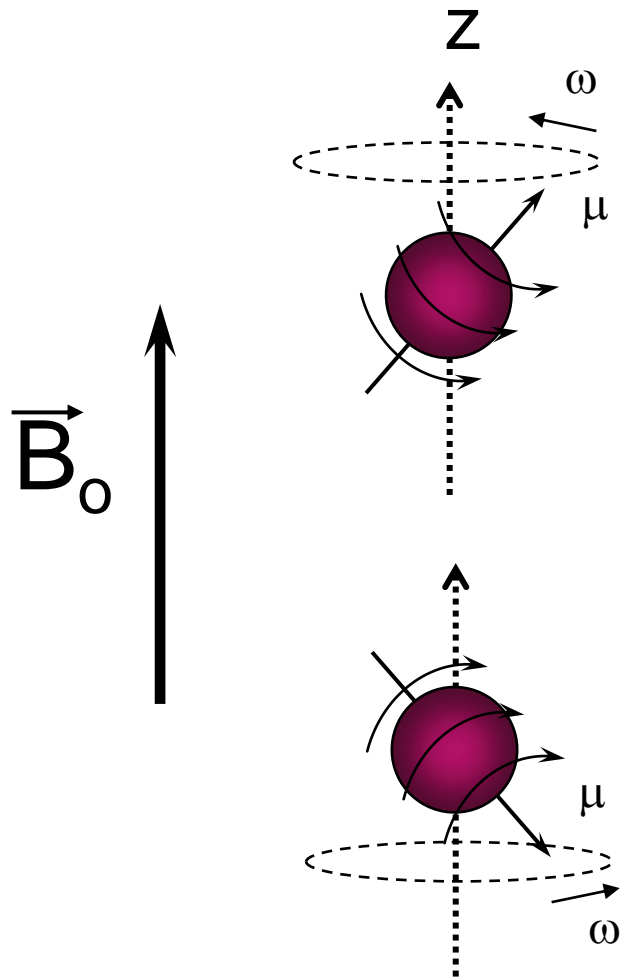B$_o$ = magnetic field strength (Telsla)

Quantum mechanical view

Energy

$\Delta E = h\nu$

B$_o$= 0   B$_o$
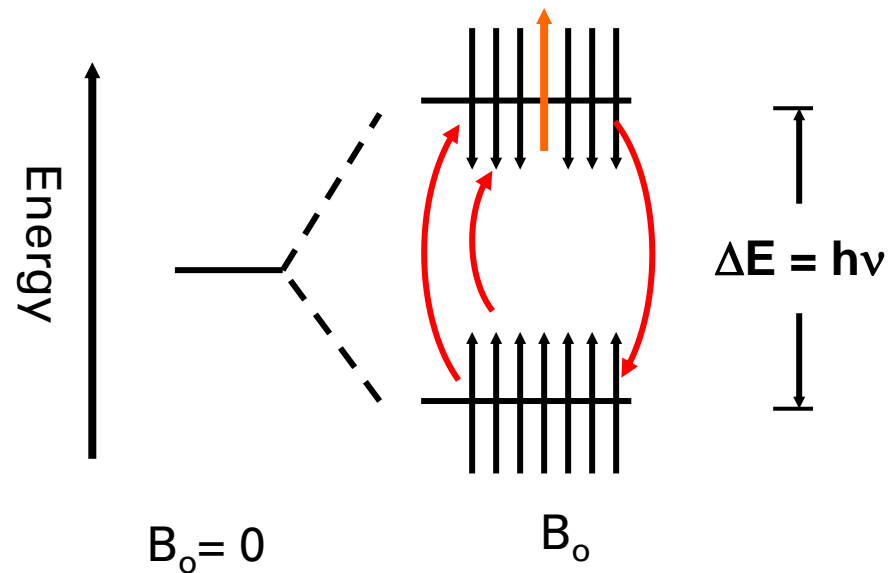
Apply an external magnetic field
(i.e., put your sample in the magnet)

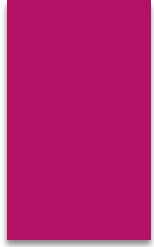$$\omega = \gamma\, B_o = \nu/2\pi$$

ω - resonance frequency
    in radians per second,
    also called Larmor frequency
ν - resonance frequency
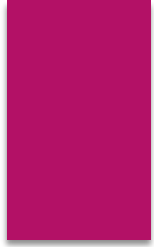   in cycles per second, Hz
γ - gyromagnetic ratio
B_o - external magnetic
    field (the magnet)

Spin 1/2 nuclei will have two orientations in a magnetic field +1/2 and -1/2.

Common NMR nuclei

- Protons, ^1H
- ^{13}C
- ^{15}N
- ^{19}F
- ^{31}P
- Sensitivity depends on natural isotopic abundance and g

  $DE = g\hbar B_0$, bigger magnet, greater sensitivity

Precession of nuclear dipoles

z

+1/2

y

M_o; net
magnetic moment
From small excess of
Nuclei in +1/2 state

M_o

x

-1/2

B_o from
magnet
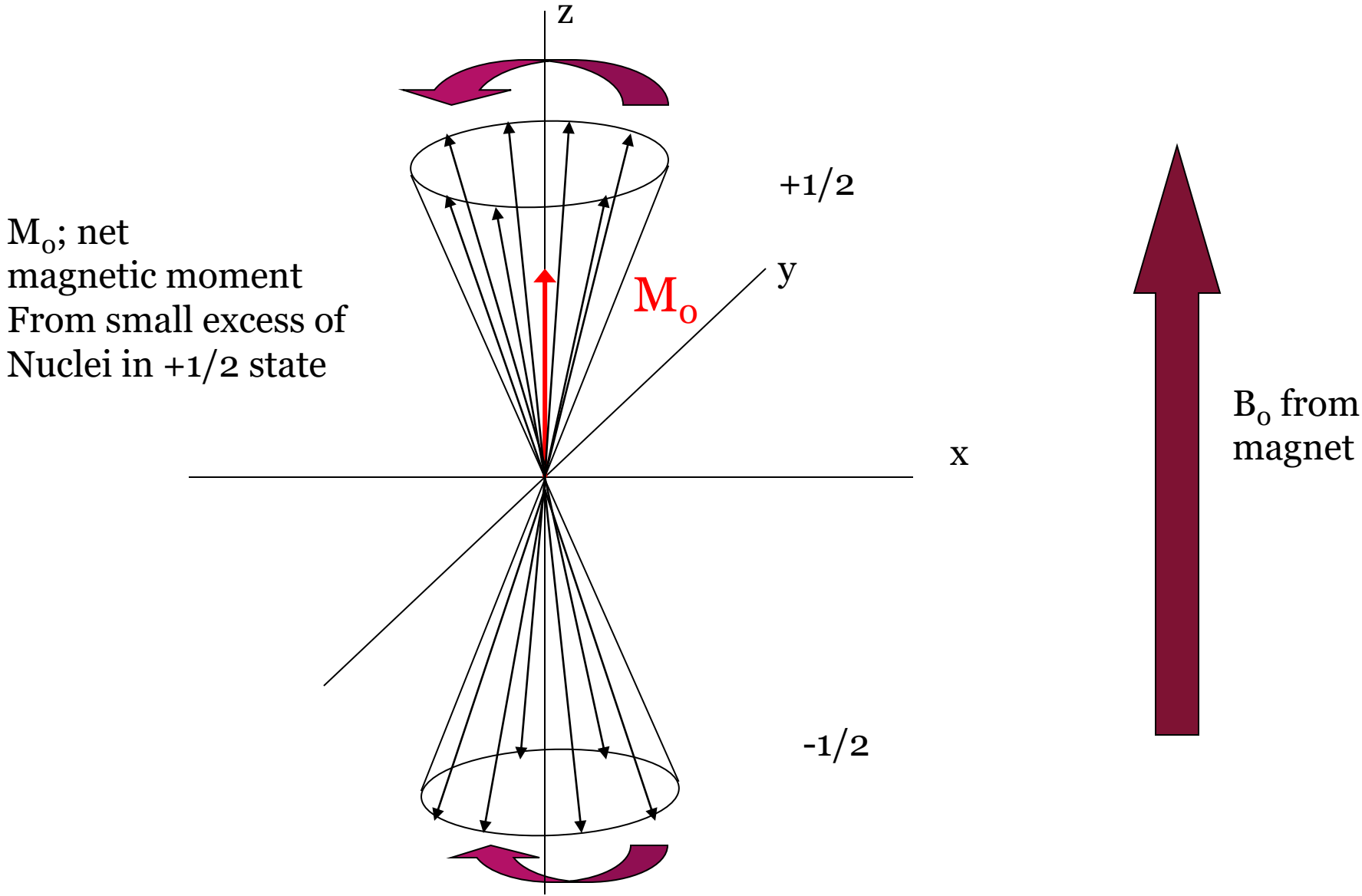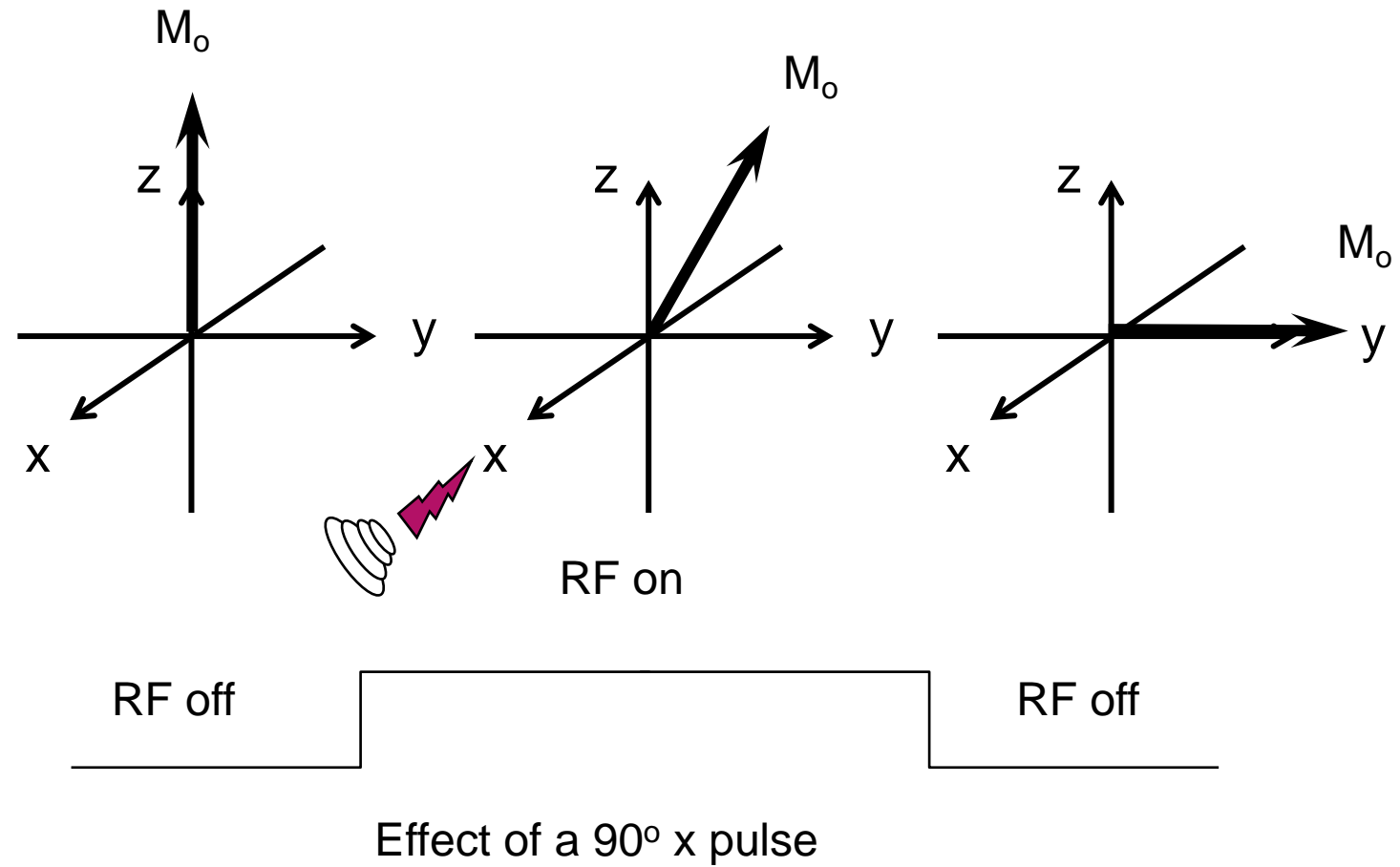
Radiofrequency generator
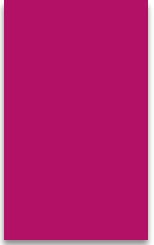
▶ A short, intense pulse generates a magnetic field in the x-y plane (excites all nuclei)

▶ M_0 of the nuclei interacts with the magnetic field produced by the pulse.
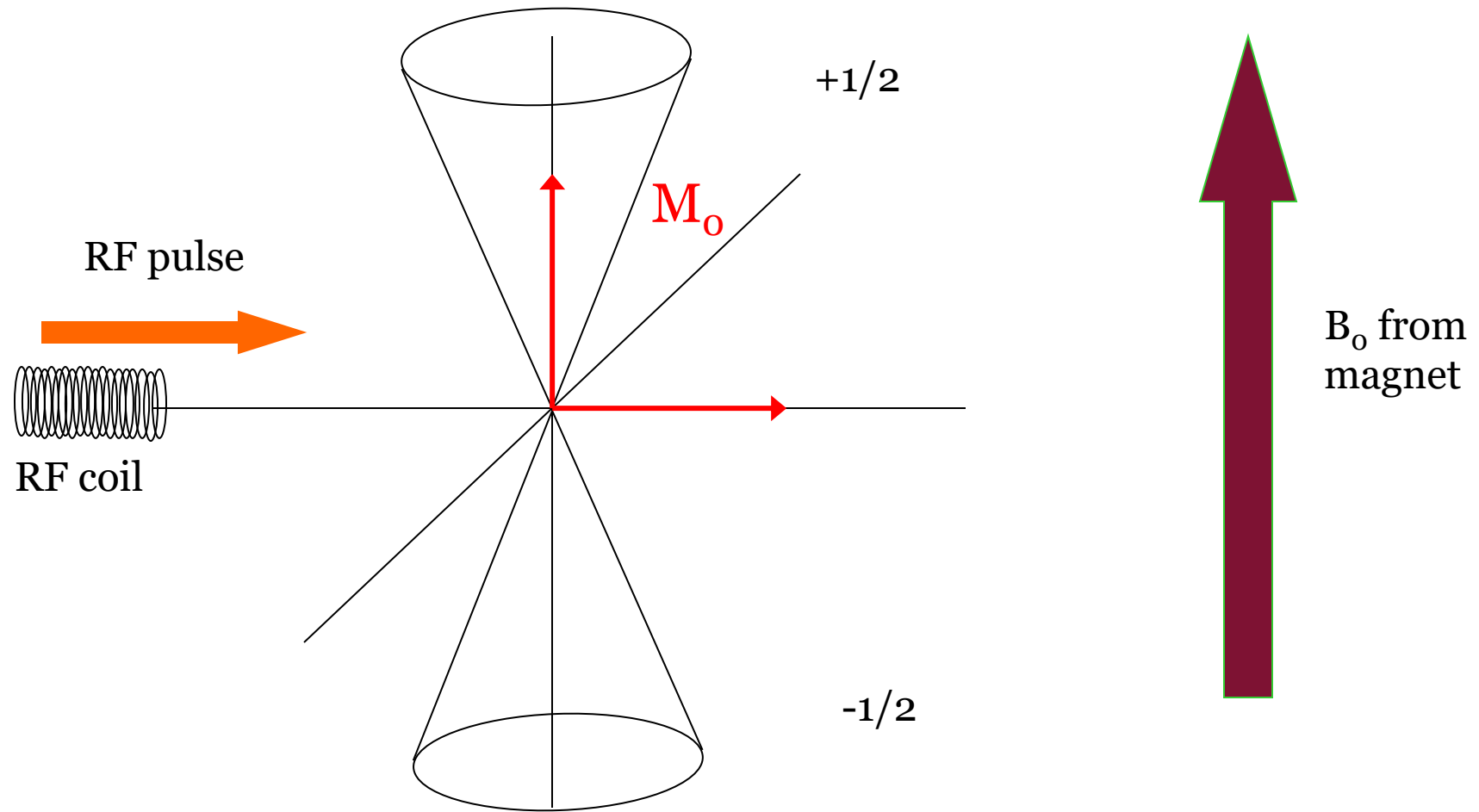
▶ Tips M_0 off axis

$\Theta = gB1t_p$

t_p – length of pulse, 90° pulse

Nuclear Spin Dynamics

Effect of a 90° x pulse

Vector Illustration of the pulse

NMR Pulse

A radiofrequency pulse is a combination of a wave (cosine) of frequency ω_o and a step function

tp

Pulse length (time, t_p)
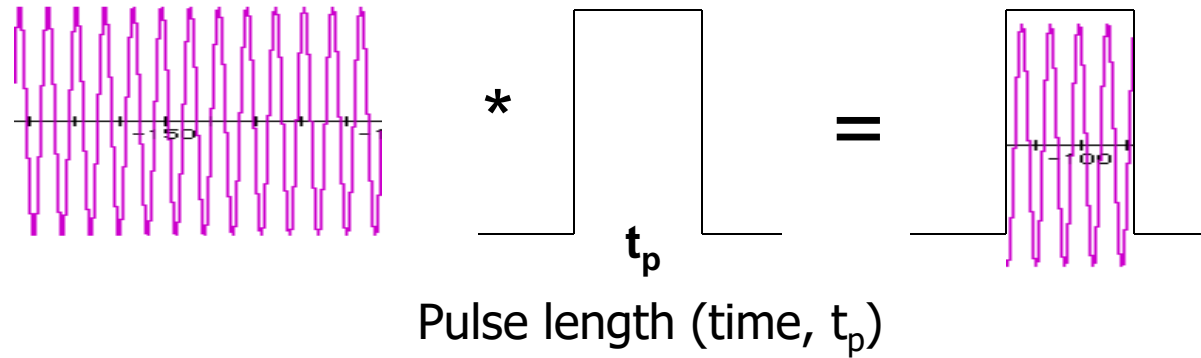
The fourier transform indicates the pulse covers a range of frequencies

FT
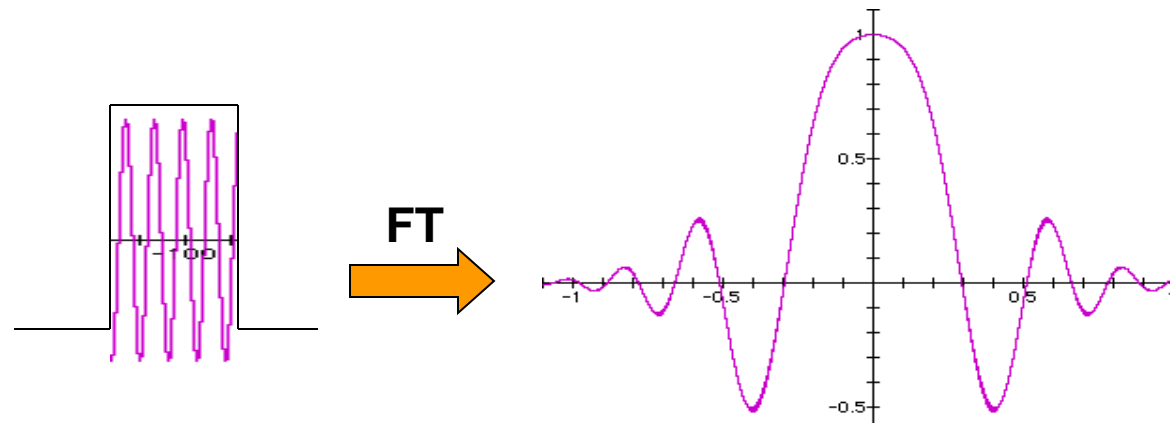
Hisenberg Uncertainty principal again: $\Delta\upsilon \cdot \Delta t \sim 1/2\pi$
Shorter pulse length – larger frequency envelope
Longer pulse length – selective/smaller frequency envelope

Sweep Width
$f \sim 1/t$

Relaxation

- T_1 spin-lattice (relaxing back to precessing about the z axis)

- T_2 spin-spin (fanning out)

(a) No B_o

Random
orientation of
magnetic dipoles

M_o
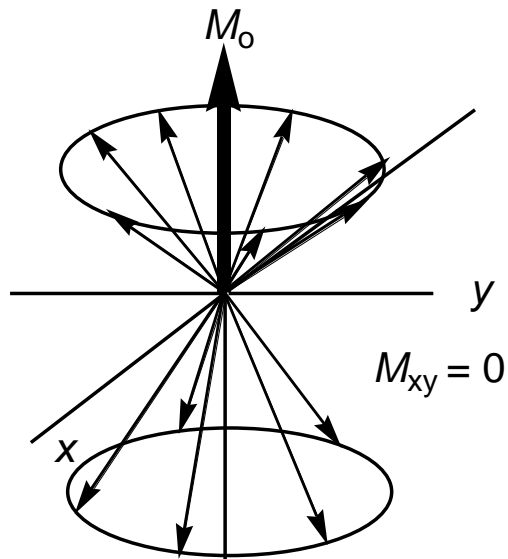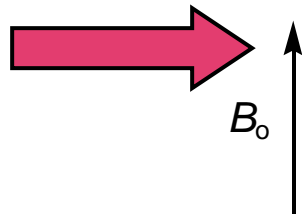
(b) B_o on; prior to resonance

Net polarization M_z is due to
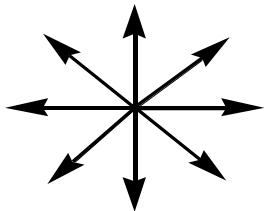population excess in higher
energy state
The magnetic vectors
precess about B_o at
the Larmor frequency ν_o

B_o

y

$M_{xy} = 0$

x

(c) At resonance $\nu_o = \nu_1$

The magnetic vectors
precess in phase with
frequency ν_1.
After resonance the return
to the equilibrium in (b)
occurs by the loss of M_{xy} via
dephasing of nuclear
dipoles by T_2 and increase
in M_z by spin inversion
due to T_1.

M

z

y

x
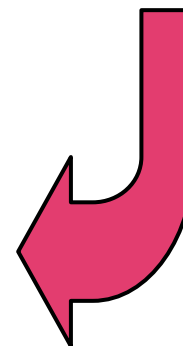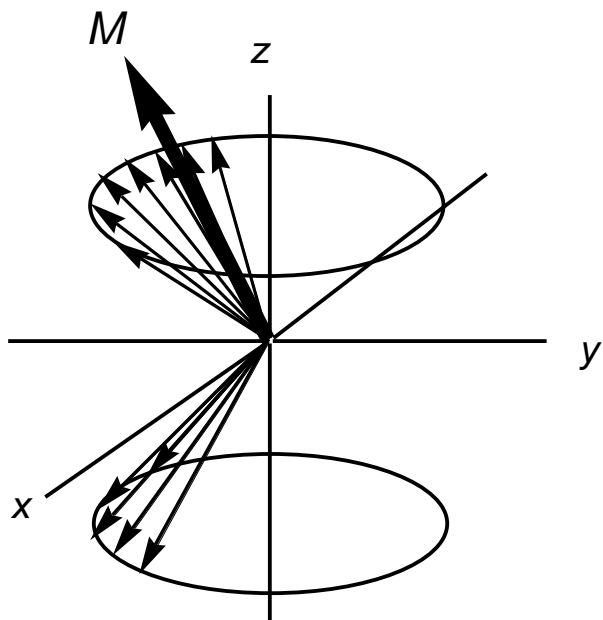
There are two primary causes of spin relaxation:
SPIN-LATTICE OR LONGITUDINAL RELAXATION

- It describes the rate of return of the Mz component of the total magnetization to equilibrium after a perturbation
- Transfer of energy to the lattice or solvent material
- Coupling of nuclei magnetic field with magnetic fields created by the ensemble of vibrational and rotational motion of the lattice or solvent.
- Results in a minimal temperature increase in sample
- Relaxation time (T_1) → exponential decay

$$M_z = M_0(1-exp(-t/T_1))$$
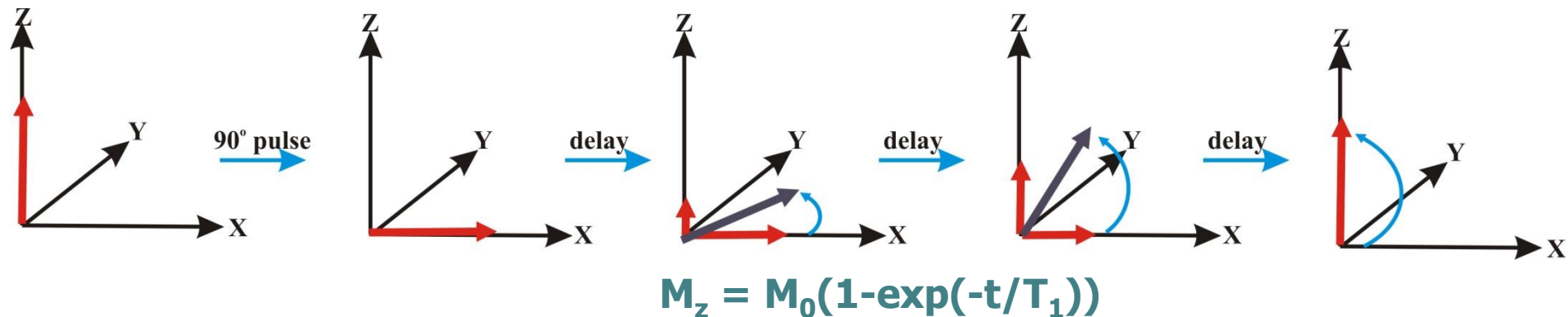
SPIN-SPIN OR TRANSVERSE RELAXATION

➤ Exchange of energy between excited nucleus and low energy state nucleus, which results in dephasing(fanning out), line broadening and signal loss

➤ Randomization of spins or magnetic moment in x,y-plane related to NMR peak line-width

➤ Relaxation time (T_2)

➤ There is no residual magnetic component in the x-y plane by the time relaxation is complete along the z axis, which means that $T_2 \le T_1$

v(1/2)
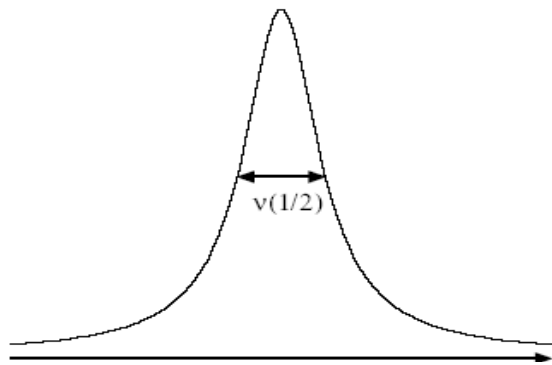
$$\nu_{1/2} = \frac{1}{\pi T_2}$$

(derived from Heisenberg uncertainty principal)

dephasing
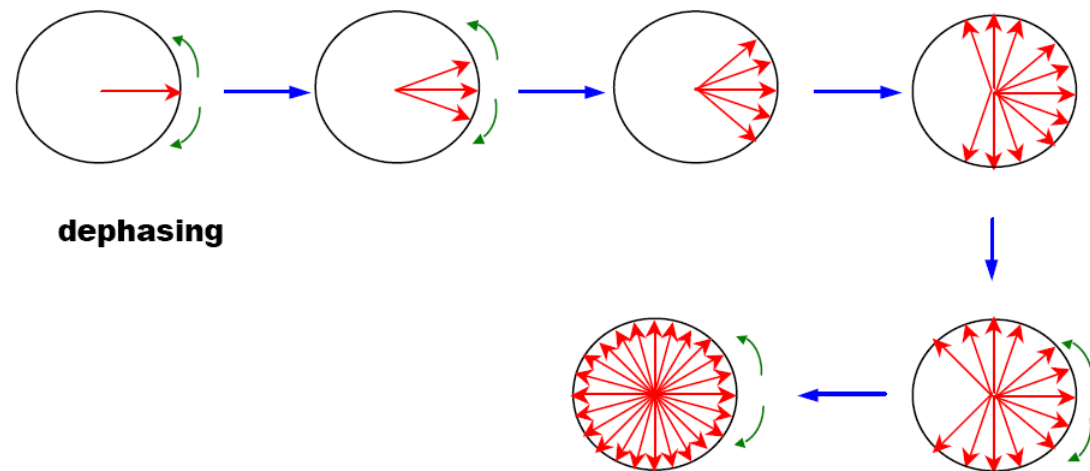
$$M_x = M_y = M_0 \exp(-t/T_2)$$

ONE-PULSE SEQUENCE

➤ *NMR pulse sequences* composed of a series of RF pulses, delays, gradient pulses and phases

➤ In a 1D NMR experiment, the FID acquisition time is the time domain (t_1)

➤ Multidimensional NMR experiments may also use multiple nuclei (^2D, ^{13}C, ^{15}N) in addition to ^1H, but usually detect ^1H

Excess of spin population along the direction of applied magnetic field.

After 90° pulse magnetization is tipped into the *xy* plane.

M=Magnetization which produces the FID. It decays as magnetization in *xy* plane diminishes after resonance

preparation

detection
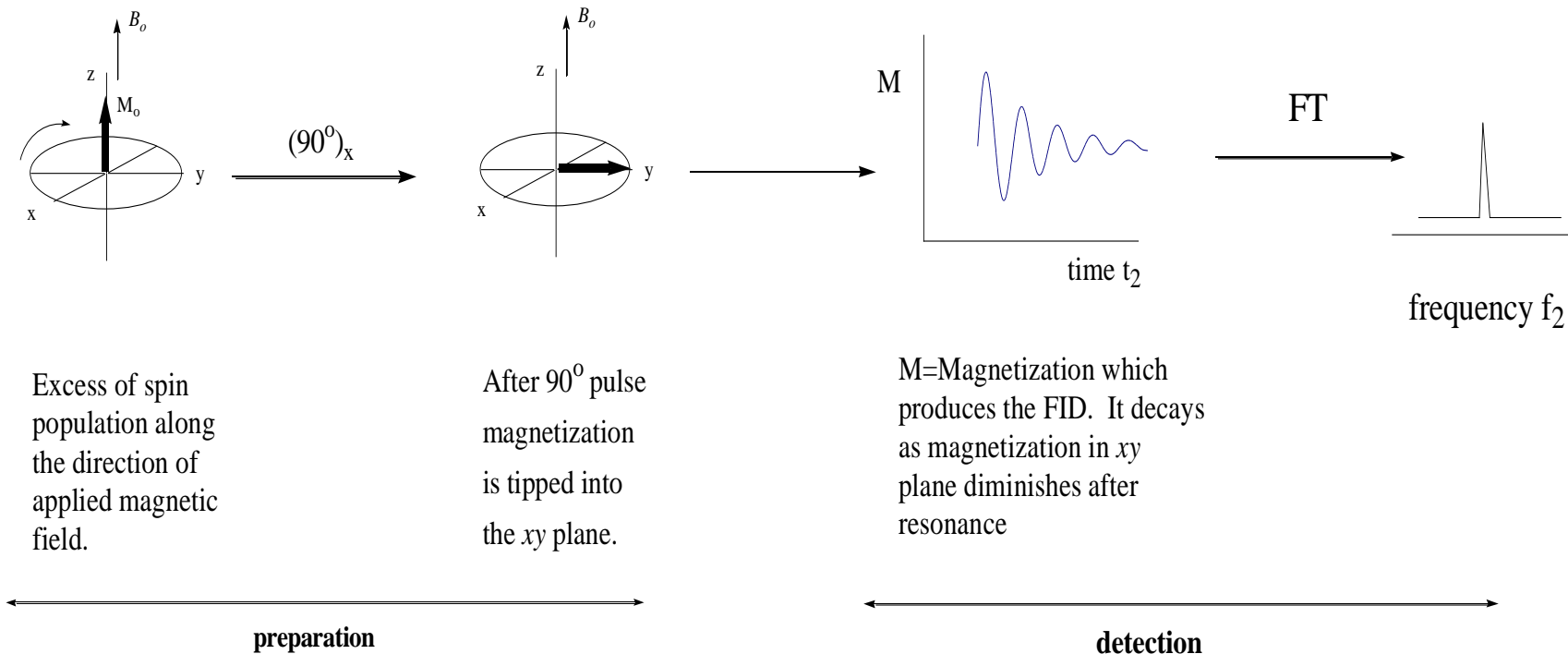
- Observed signal decays as a function of T_2 relaxation
- peak width at half-height ($n_{1/2}$) is related to T_2
- NMR signal is collected in Time domain, but prefer frequency domain
- Transform from the time domain to the frequency domain using the Fourier function
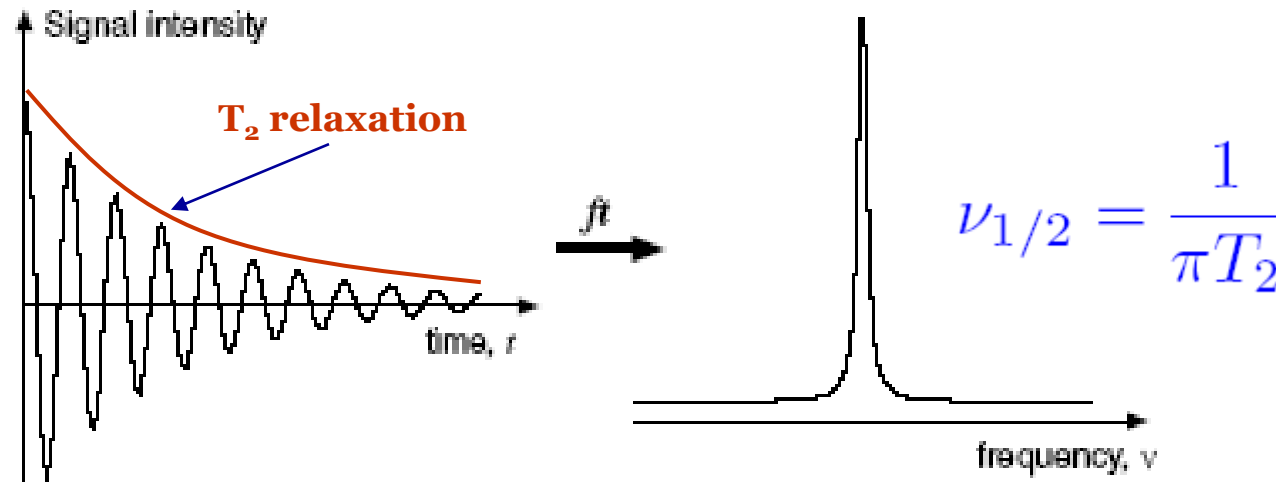

$$F(\nu) \propto \int_{-\infty}^{\infty} f(t)e^{-i2\pi\nu t}\, dt$$

Fourier Transform is a mathematical procedure that transforms time domain data into frequency domain

➤ Conventional NMR spectra (one-dimensional spectra) are plots of intensity *vs. frequency;* 1D peaks tell you something about a particular chemical site: what it's chemical environment is like, how many nuclei are present, how many nuclei are near the site, etc. But there's no mechanism for telling you anything about the connections.

➤ *In two-dimensional spectroscopy intensity is plotted* as a function of two frequencies, usually called *F1 and F2. There are various* ways of representing such a spectrum on paper, but the one most usually used is to make a contour plot in which the intensity of the peaks is represented by contour lines drawn at suitable intervals, in the same way as a topographical map.

➤ The position of each peak is specified by two frequency co-ordinates corresponding to *F1 and F2. Two-dimensional NMR spectra* are always arranged so that the *F2 co-ordinates of the peaks correspond to* those found in the normal one-dimensional spectrum, and this relation is often emphasized by plotting the one-dimensional spectrum alongside the *F2* axis.

THE BASIC IDEA OF 2D NMR
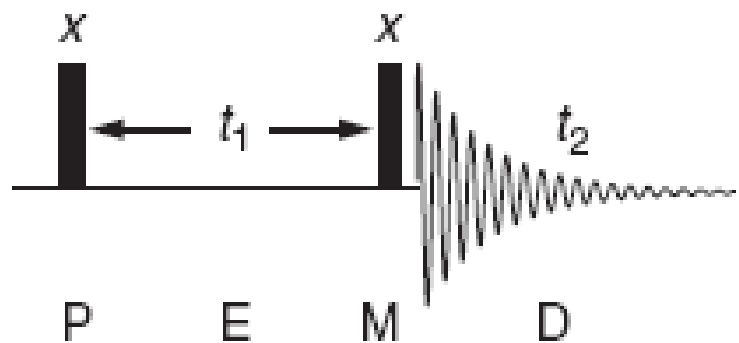
P: Preparation period
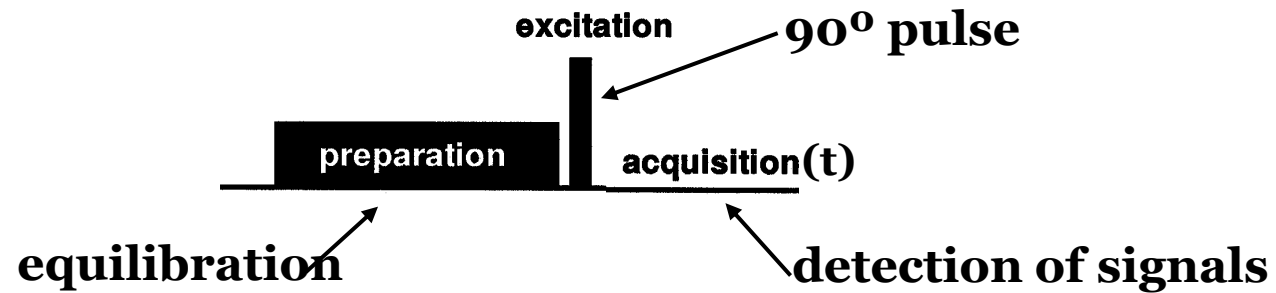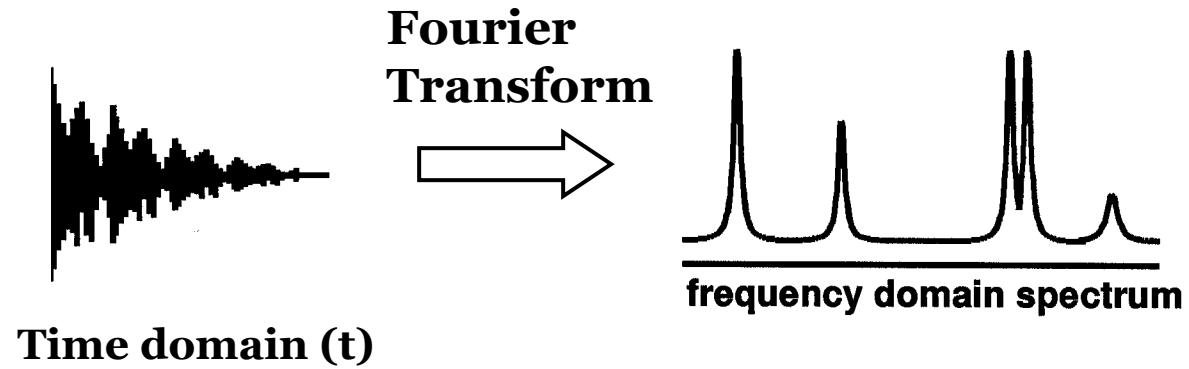E: Evolution period
M: Mixing period
D: Acquisition period

Stack plot

Preparation	Evolution t_1	Mixing τ	Acquisition t_2
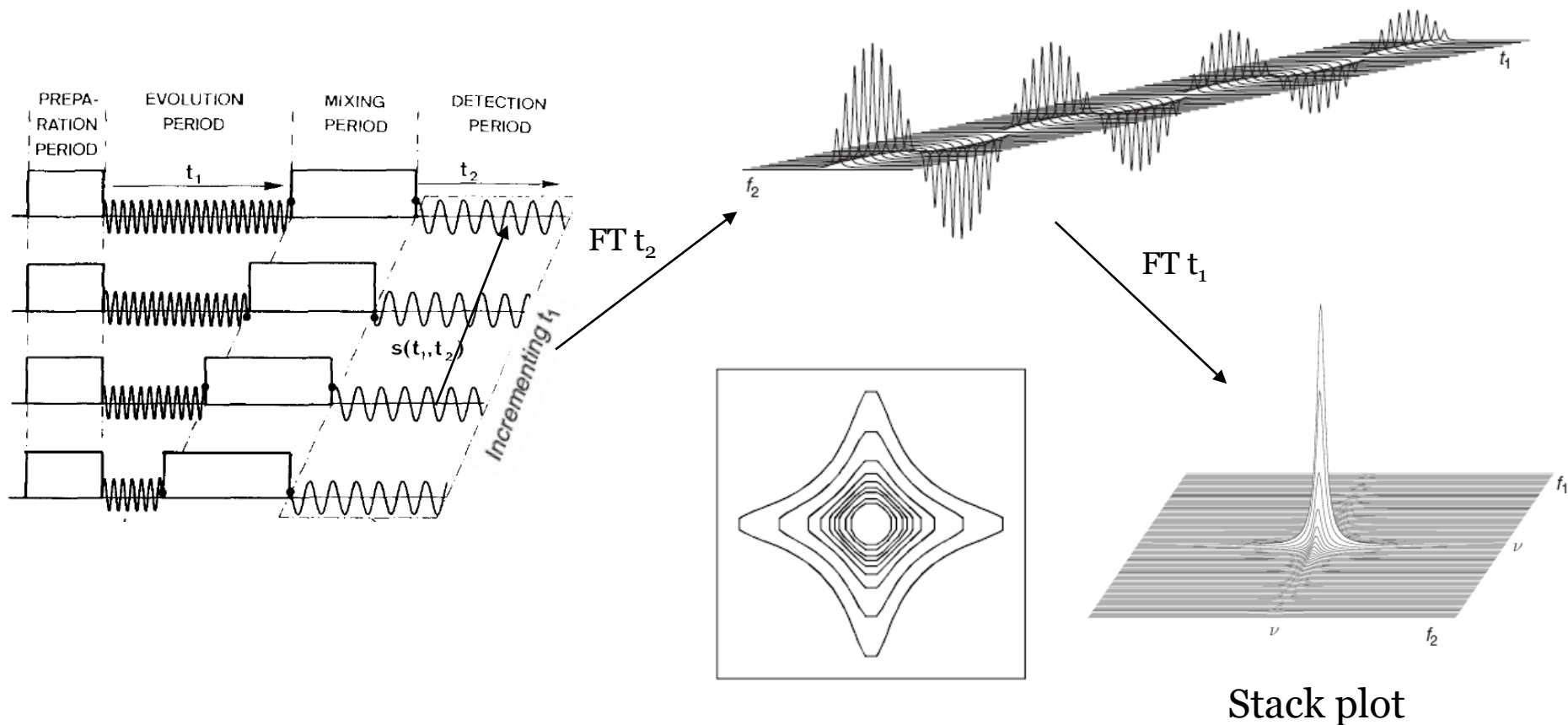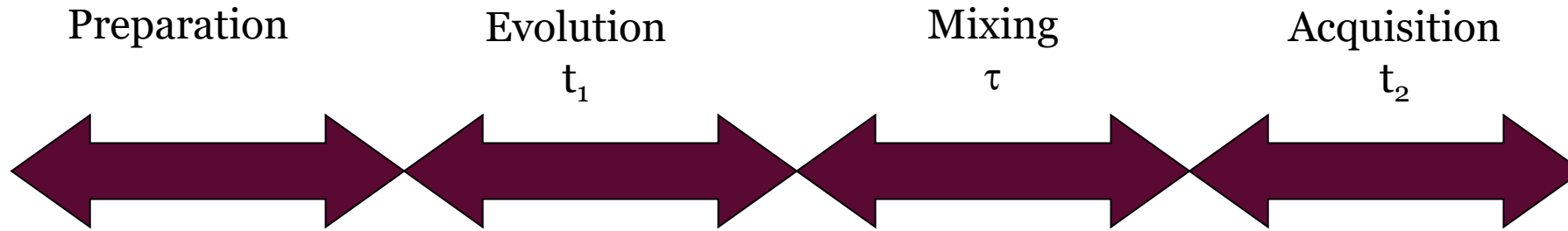
➤**Preparation**: Initial pulse to set up the spin system for the desired nonequilibrium state.

➤**Evolution**: There is a variable delay t_1, which allows some part of the spin system to evolve (i.e. couplings, chemical shift).

➤**Mixing**: Coherence is transferred from one spin to another to establish correlation between the two dimensions. Usually some combinations of pulses and delays.

➤**Acquisition**: The FID is recorded in the conventional fashion.
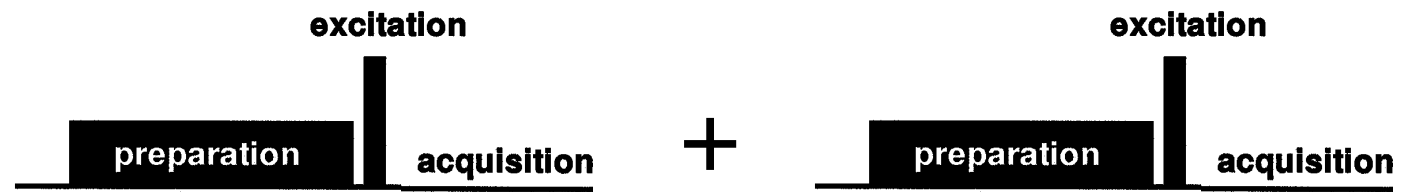
The Pulse FT NMR Experiment

Experiment

excitation

90° pulse

preparation

acquisition (t)

equilibration

detection of signals

Data Analysis

Fourier Transform

Time domain (t)

frequency domain spectrum

The 2D NMR Pulse Sequence

excitation

preparation | acquisition

+

excitation

preparation | acquisition

1D + 1D = 2D

excitation

preparation | evolution (t_1) | mixing | acquisition (t_2)

[2nd preparation]
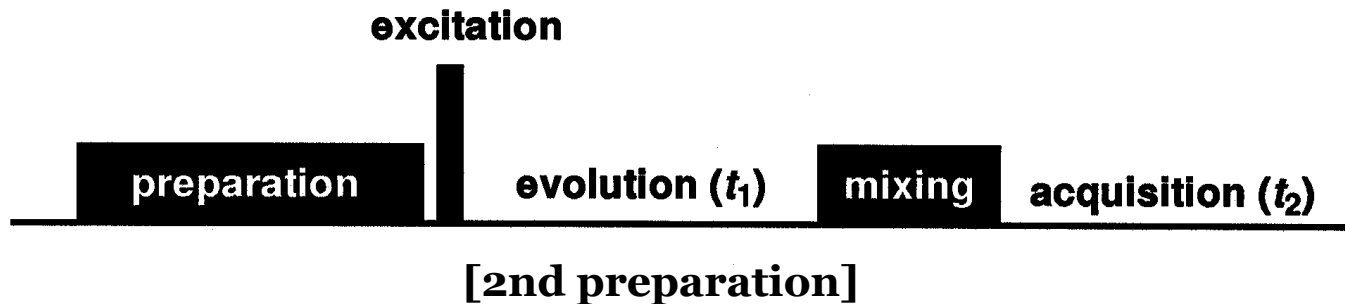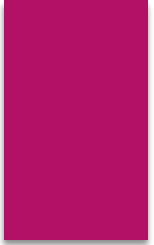
❖ **2D NMR essentially allows us to irradiate all of the chemical shifts in one experiment and gives us a matrix or two dimensional maps of all of the affected nuclei.**

❖ **Two dimensional NMR spectroscopy can be defined as a spectral method in which the data are collected in two different time domains**

❖ **Acquisition of the free induction decay, FID (t_2), and a successively incremented delay (t_1) to elapse before detection, then during this time interval (the evolution period) the nuclei can be made to interact with each other in various ways, depending on pulse sequences applied.**

❖ **The resulting FID is accordingly subjected to two successive sets of Fourier transformations to furnish a two-dimensional NMR spectrum in two frequency axes.**

❖ **The major difference between one and two- dimensional NMR methods is therefore the insertion of an evolution time, t_1.**
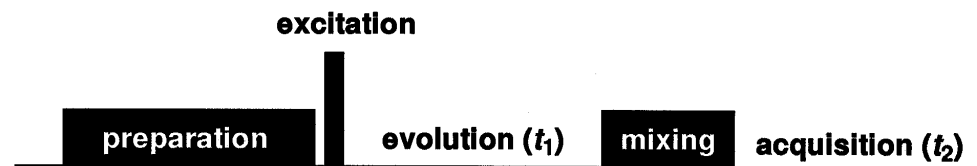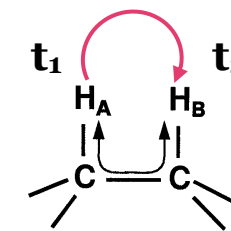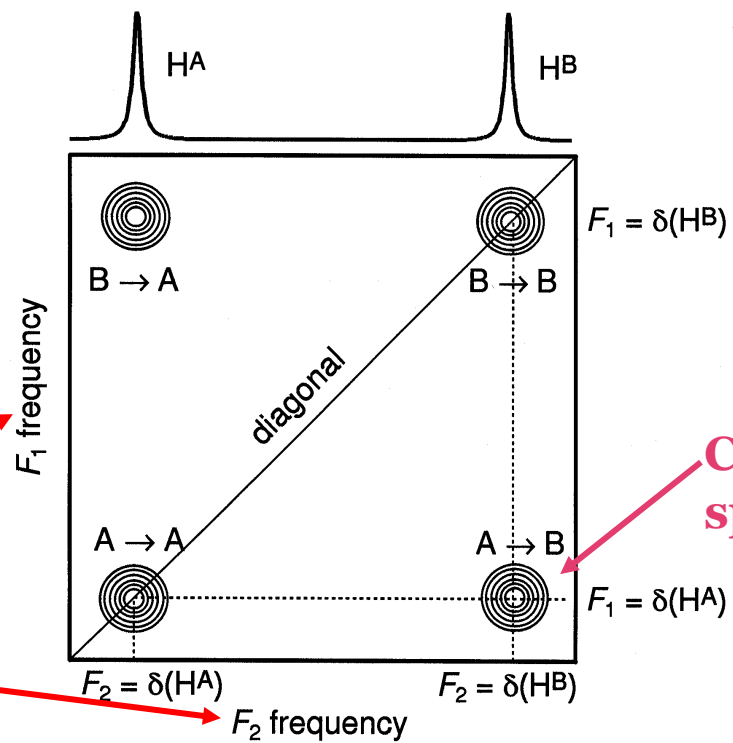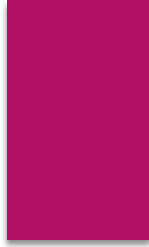
2D NMR: Coupling is the Key
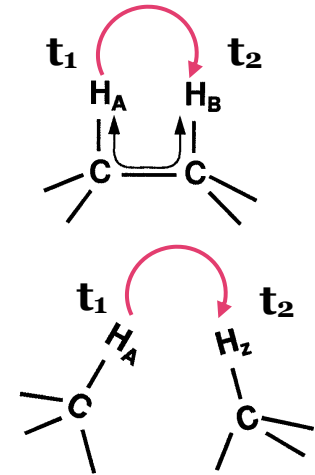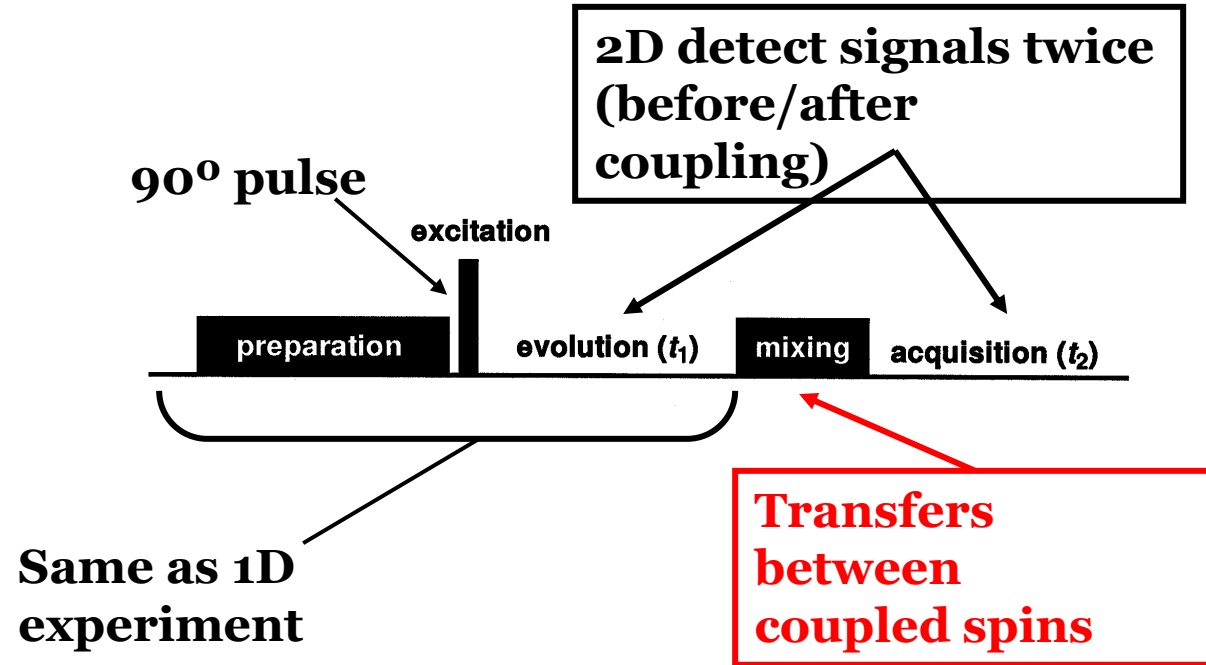
The 2D NMR Spectrum
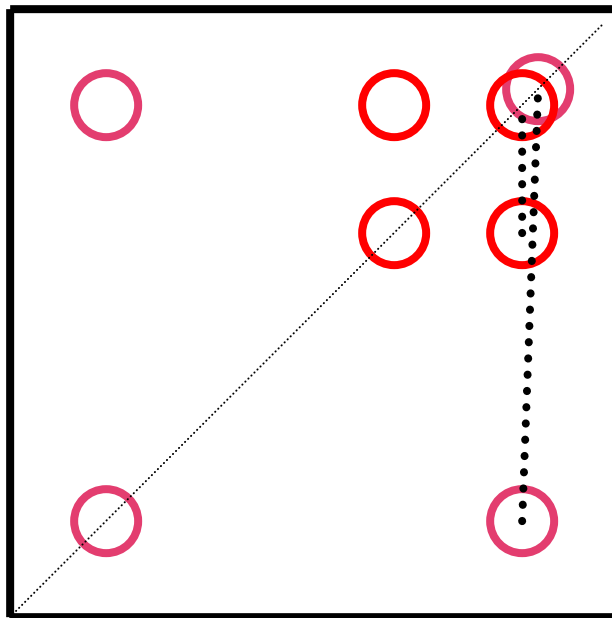
The Power of 2D NMR:
Resolving Overlapping Signals

1D

2 signals overlapped

2D

2 cross peaks resolved

n is the number of increments

PROCESSING 2D DATA

❖ All 2D experiments are a simple series of 1D experiments collected with different timing.

❖ In general, 2D's can be divided into two. types, homonuclear and heteronuclear .

❖ Each type can provide either through-bond (COSY-type) or through space (NOESY-type) coupling information.

❖ A 2D frequency correlation map is produced after a Fourier transform in both dimensions (t_1 and t_2).

❖ On modern spectrometers, only the proton 90 degree pulse width needs to be determined to run a full series of 2D experiments.

Technique	Principal applications
COSY-90	Correlating coupled homonuclear spins. Typically used for correlating protons coupled over two or three bonds but may be used for any high-abundance nuclide. The basic COSY experiment
DQF-COSY	Correlating coupled homonuclear spins. Typically used for correlating protons coupled over two or three bonds. Higher-resolution display than basic COSY. Additional information on magnitudes of coupling constants may be extracted from 2D peak fine structure. Singlets suppressed
COSY-β	Correlating coupled homonuclear spins. Typically used for correlating protons coupled over two or three bonds but may be used for any high-abundance nuclide. Reduced 2D peak structure over basic COSY. Vicinal and geminal coupling relationships can be differentiated in some cases
Delayed COSY	Correlating coupled homonuclear spins through small couplings. Often used to identify proton correlations over many bonds (>3), hence also known as long-range COSY
TOCSY	Correlating coupled homonuclear spins and those that reside within the same spin system but which may not share mutual couplings. Employs the propagation of magnetisation along a continuous chain of spins. Powerful technique for analysing complex proton spectra
INADEQUATE	Correlating coupled homonuclear spins of low natural abundance (<20%). Typically used for correlating adjacent carbon centres at natural abundance but has extremely low sensitivity
ADEQUATE	Correlating coupled homonuclear spins of low natural abundance, primarily ^{13}C–^{13}C, but employing ^{1}H excitation and detection for sensitivity improvement

TYPES OF 2D NMR EXPERIMENTS

- AUTOCORRELATED
  - Homonuclear J resolved
  - ^1H-^1H COSY
  - TOCSY
  - NOESY
  - ROESY
  - INADEQUATE

- CROSS-CORRELATED
  - Heteronuclear J resolved
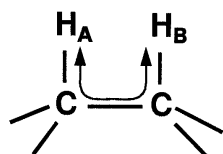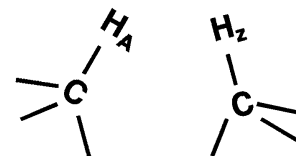 - ^1H-^{13}C COSY (HETCOR)
  - HMQC
  - HSQC
  - HMBC

Scalar Coupling

Dipolar Coupling

Types of Correlation Spectroscopy

Name	F1 nucleus	Mixing	F2 nucleus
COSY	^1H	J	^1H
TOCSY	^1H	$J\ J\ J$	^1H
NOESY	^1H	NOE	^1H
HETCOR	^1H	$^1J\ CH$	^{13}C
HMQC	^{13}C	$^1J\ CH$	^1H
HMBC	^{13}C	$^{2,\ 3}J\ CH$	^1H