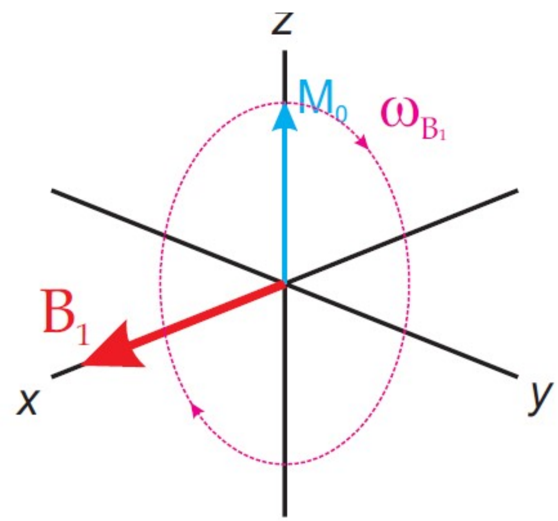
Introduction to Multidimensional NMR: Recap NMR principles and 1D

How can we move the magnetization off the Z-axis?

If we could apply a ***second*** magnetic field that is perpendicular to the z-axis, we would perturb the equilibrium by forcing a precession. This ω_{B_1} precession frequency is directly proportional to the applied B_1 field strength.



$$\omega_{B_1} = \gamma B_1$$

How do we physically do this?

Answer: We use the magnetic component of a radio frequency (RF) wave. (Remember electro-magnetic radiation has a magnetic component.) This RF irradiation is introduced to a sample by a coil that is wound perpendicular to main field (B_0).

This RF has to be at or near the resonance frequency to have an effect.

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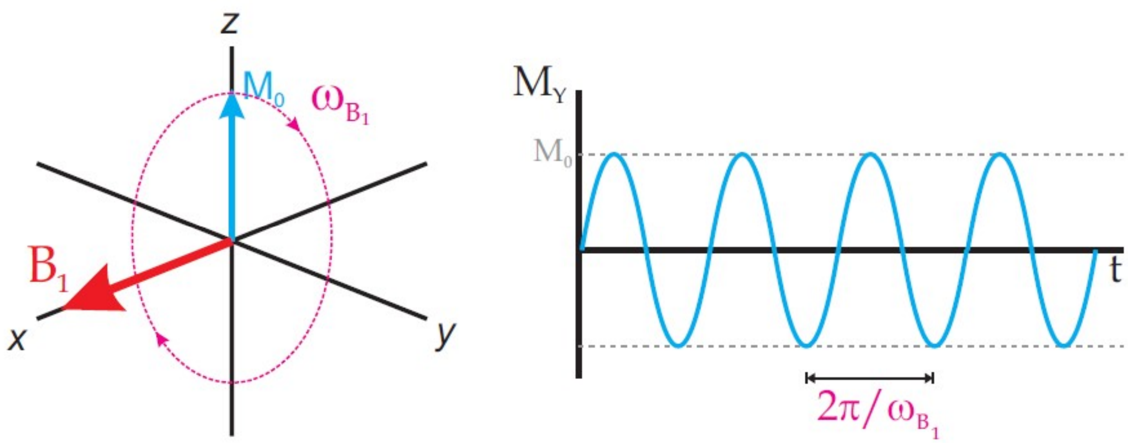
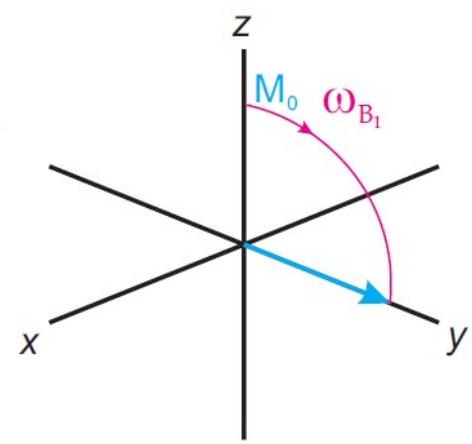
If we ignore relaxation, the y component of the magnetization will simply oscillate at frequency ω_{B_1} when B_1 is applied along the x-axis.



We can control the final orientation of the magnetization by precisely setting the duration of the irradiation field.

If:

$t_p = \pi/2\omega_{B_1} \equiv 90°$ Pulse Width



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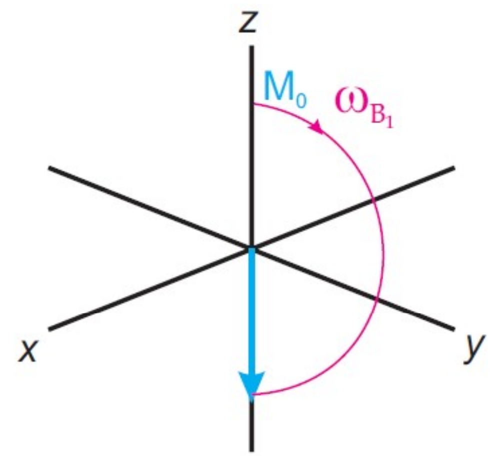
Other orientations are possible:

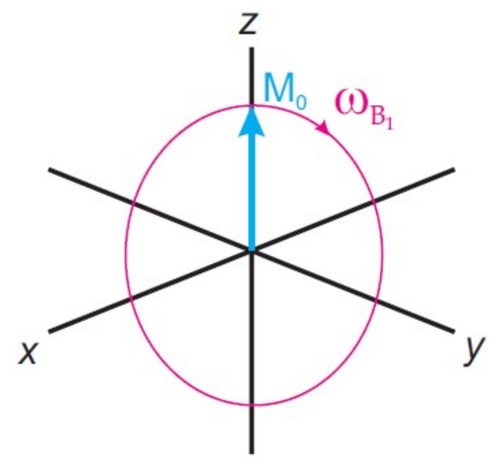
If:

$t_p = \pi / \omega_{B_1} \equiv 180°$ Pulse Width



If:

$t_p = 2\pi / \omega_{B_1} \equiv 360°$ Pulse Width



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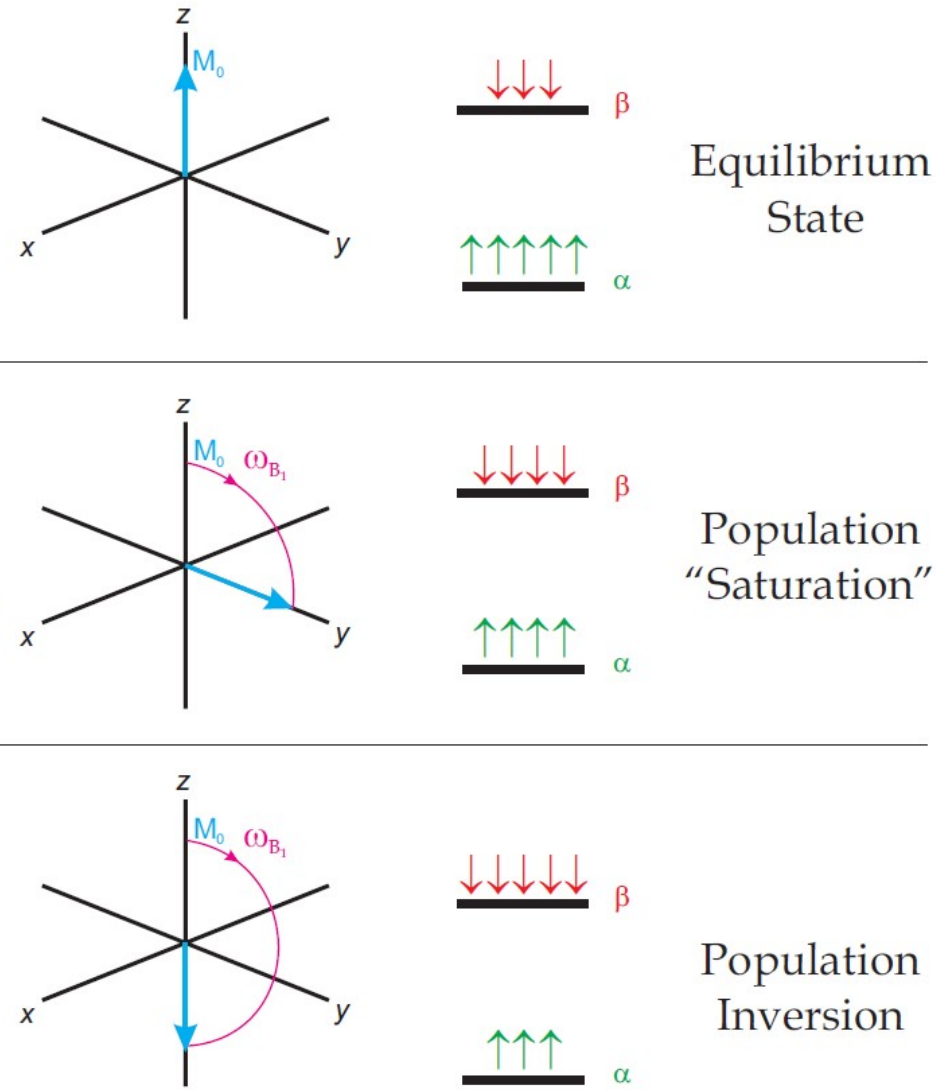
What do pulses do to the populations of the energy levels?

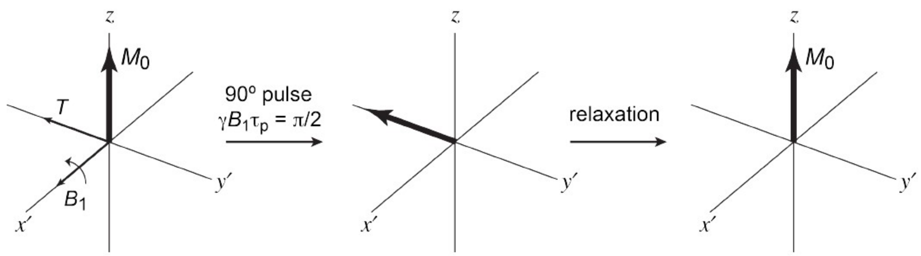
Note: Energy levels cannot provide any insight to the magnetization in the xy plane.



Introduction to Multidimensional NMR:

Recap NMR principles and 1D

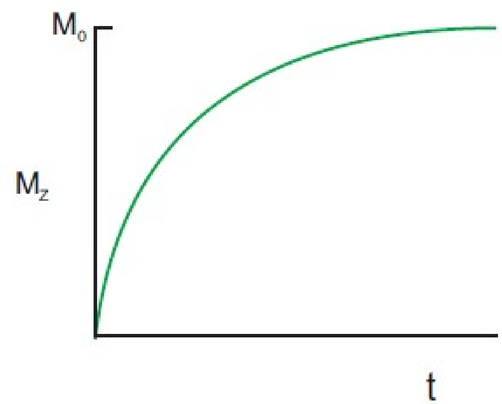
In these forms the Bloch equations show how magnetization reaches equilibration after a perturbation:

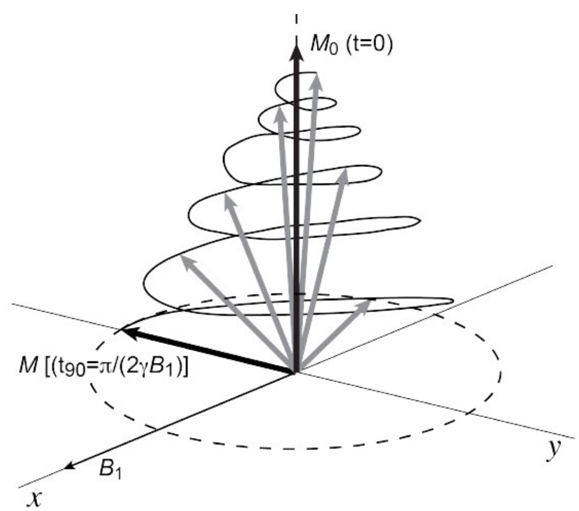


With time, normal relaxation processes return the system to thermal equilibrium, and M_0 returns to the +z-axis

The Z-component:

$$M_Z = M_0 (1 - e^{(-t/T_1)})$$





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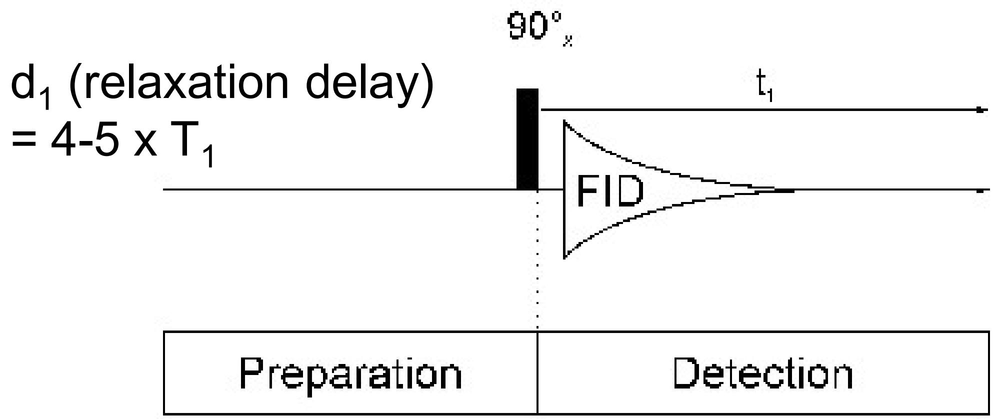
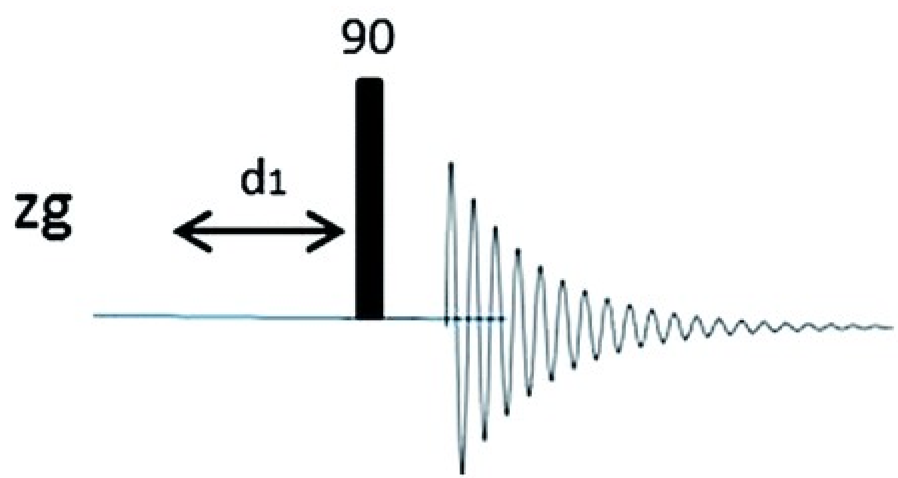
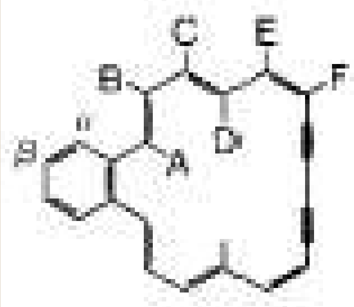
Recap NMR principles and 1D

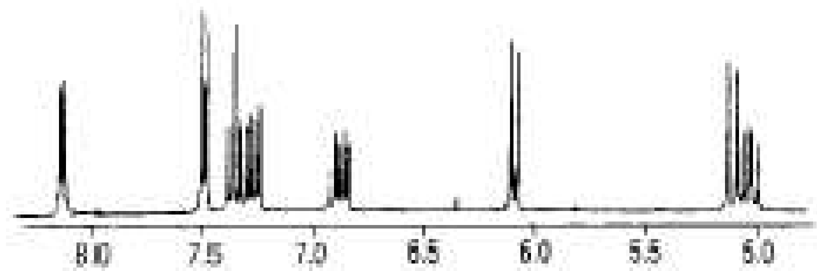




The limitation of 1D NMR is solved by nD

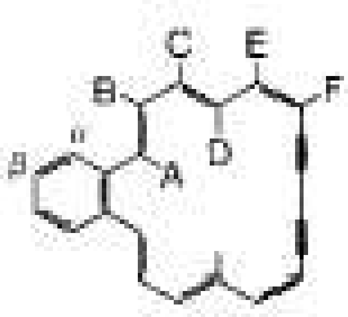


1D ^{1}H NMR



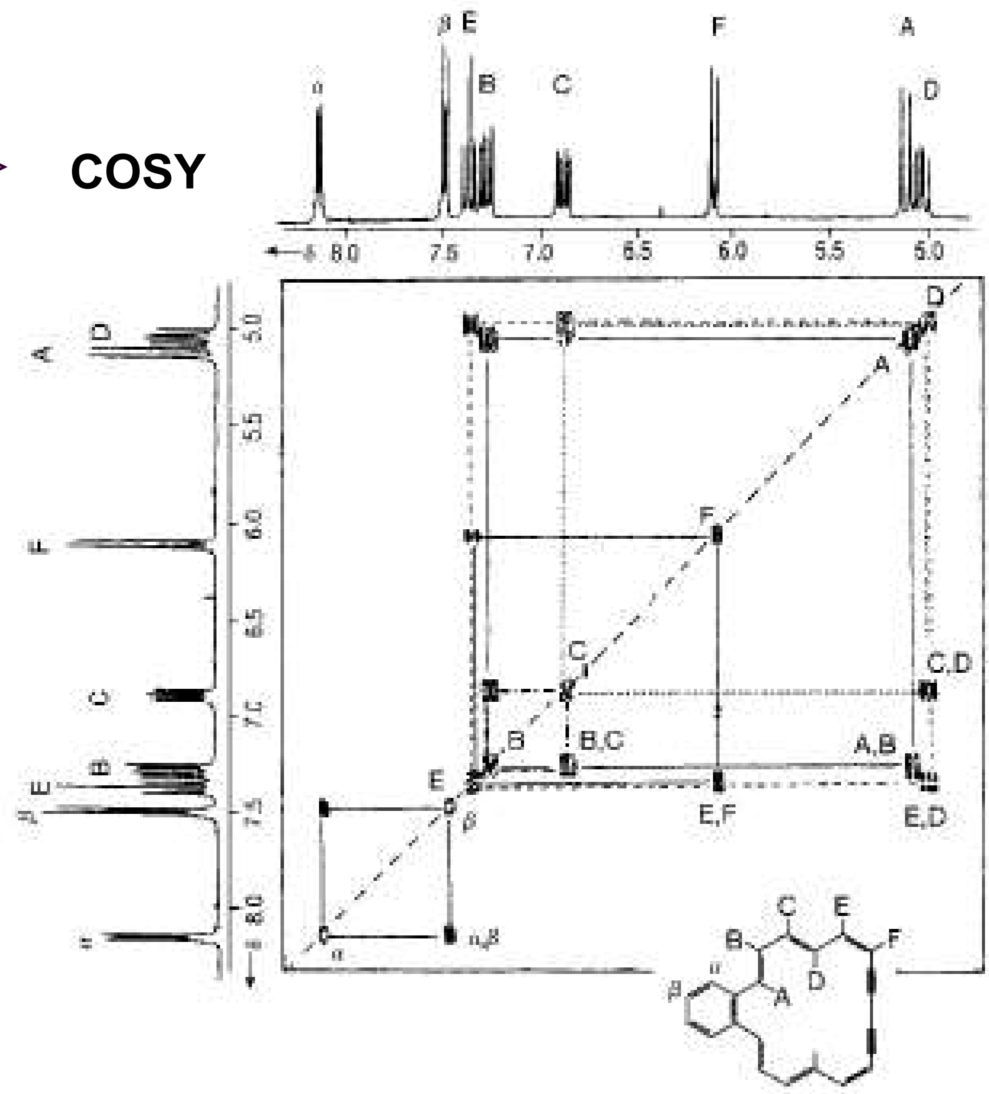
- How to assign ^{1}H chemical shifts?
- What are the coupled ^{1}H nuclei?

The importance of 2D NMR in structure elucidation



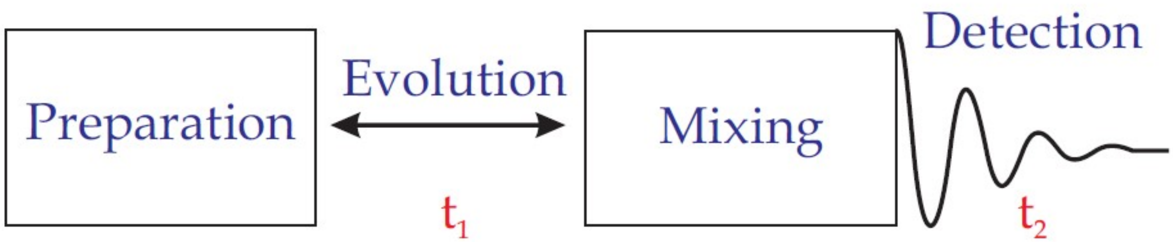
2D ^{1}H-^{1}H NMR

COSY



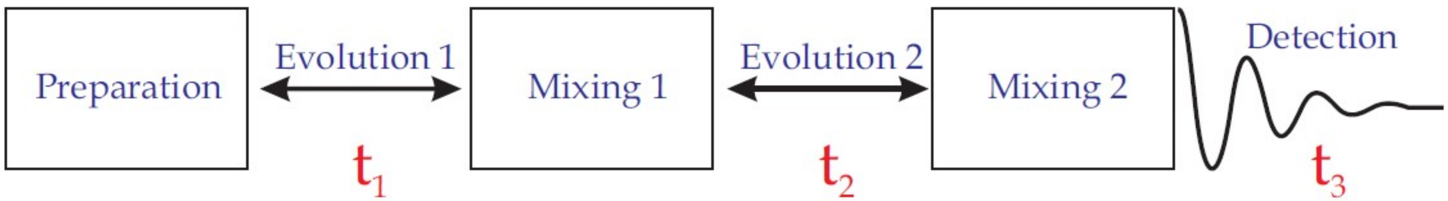
General scheme of 2D and nD NMR

The general form of any 2D NMR sequence is:

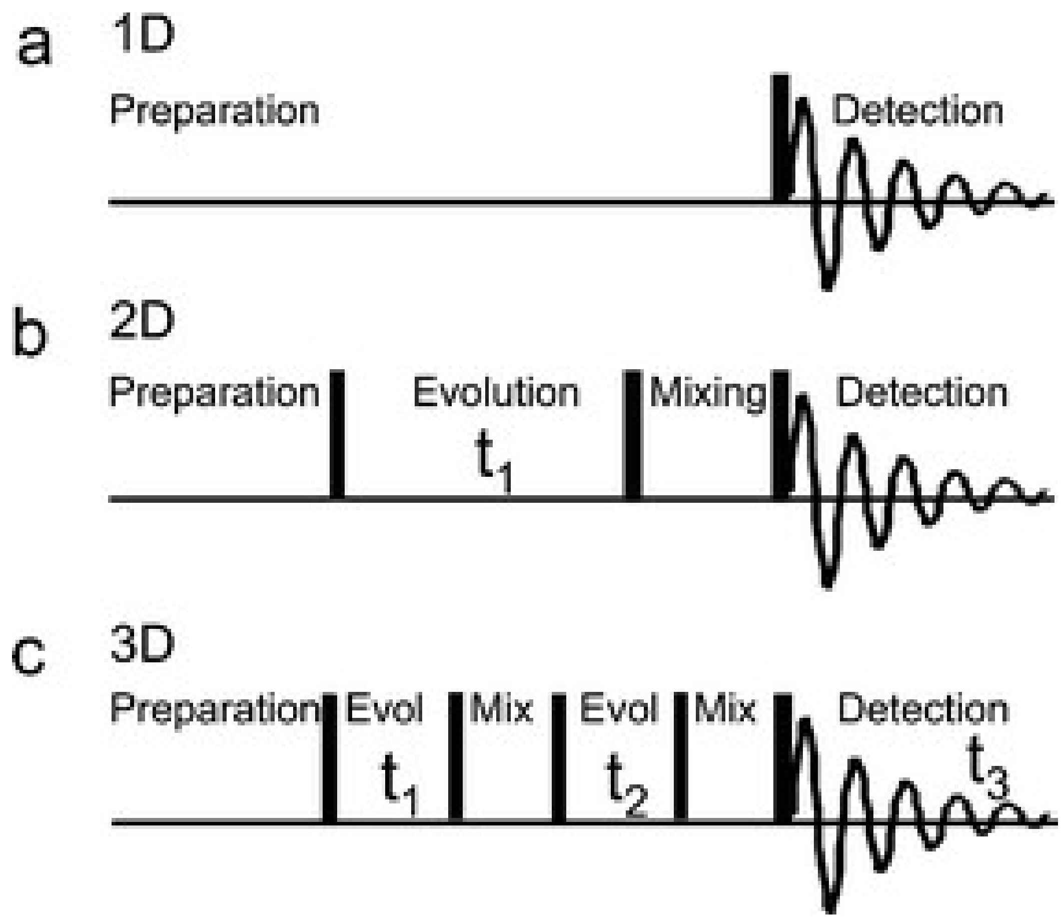


The Preparation and Mixing periods are a pulse or series of pulses and **fixed** delays. The Evolution period is the varying delay time (t_1) where chemical shift or J modulation occurs. The Detection period (t_2) is similar to the collection of 1D FIDs.

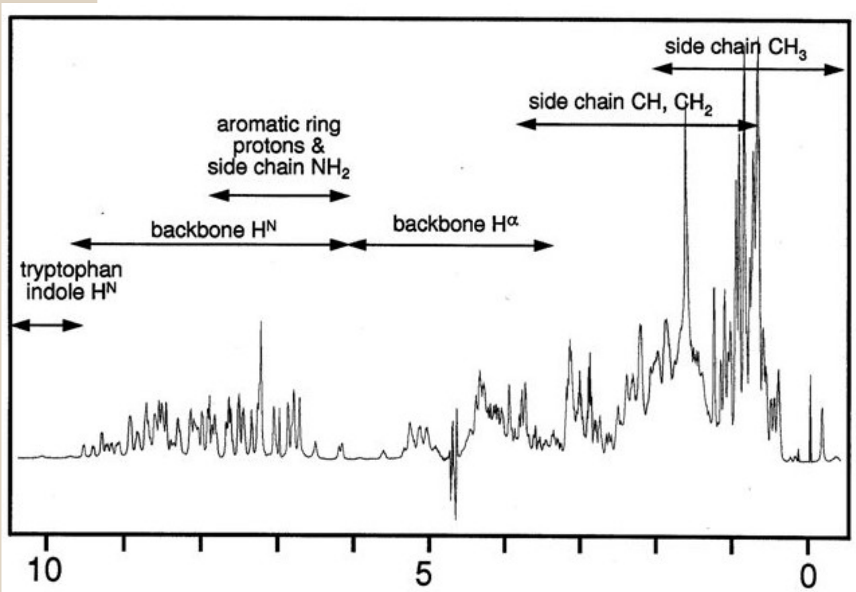
Taken further, nD NMR can have three or more time domains in the general form:

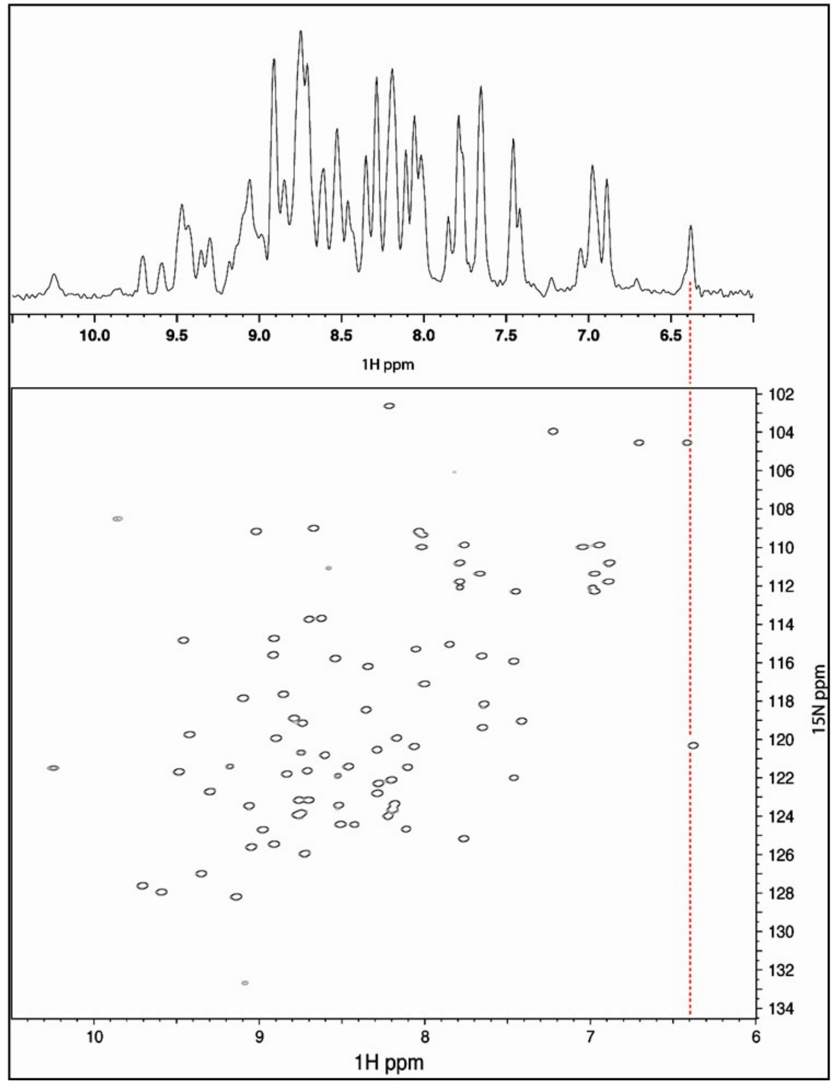


Pulse sequences of 1D, 2D and 3D NMR

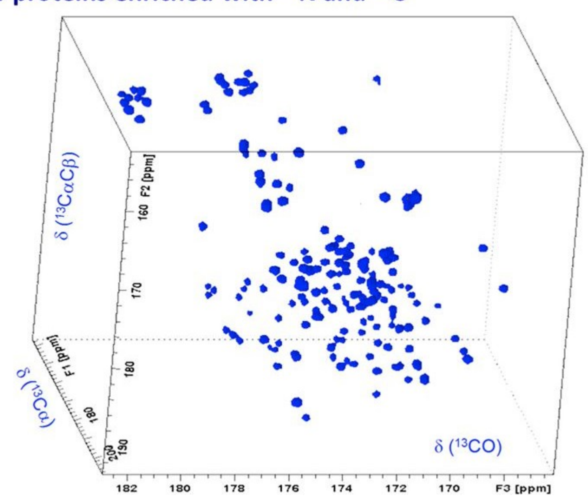


Example: 1D, 2D and 3D NMR spectra of protein



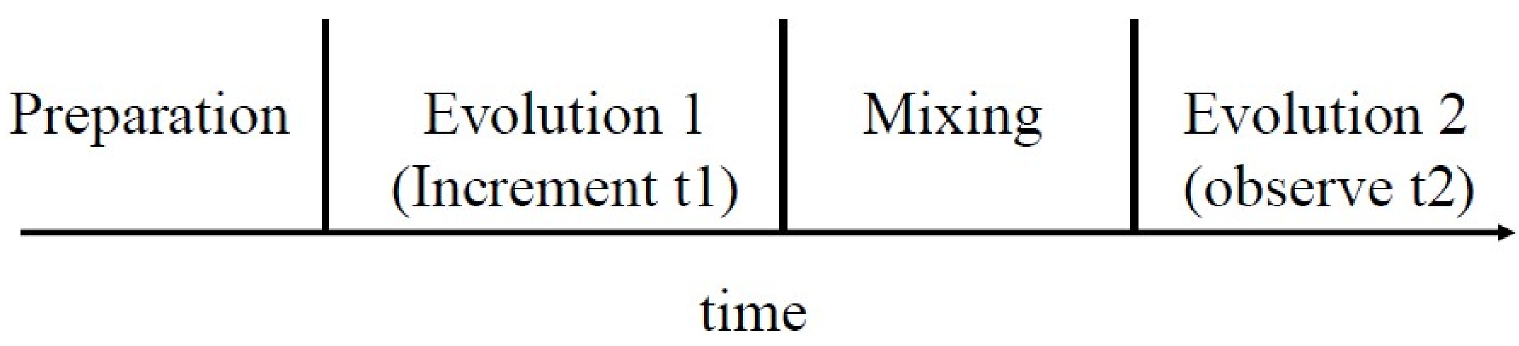


For proteins enriched with ^{15}N and ^{13}C

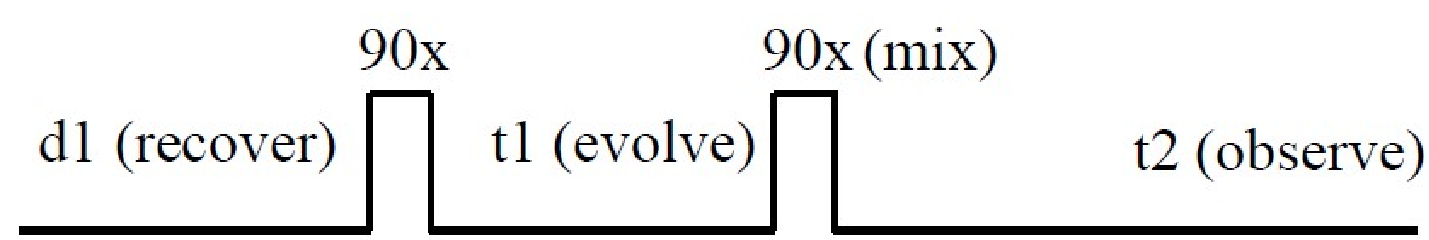


General scheme of 2D, nD NMR and ^1H-^1H COSY

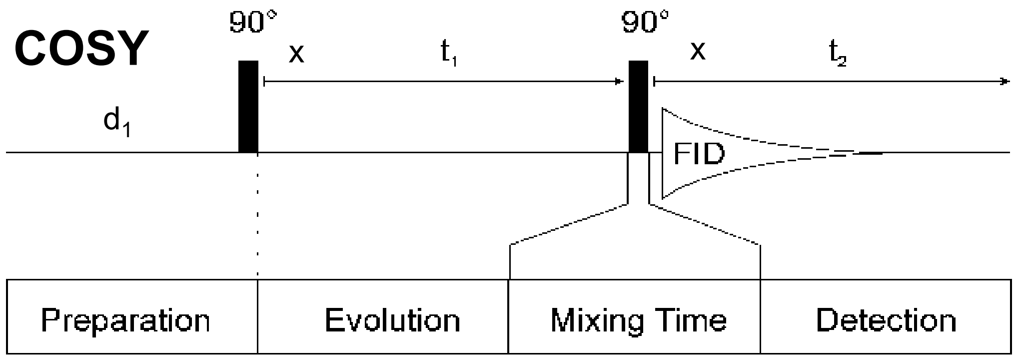
A General Scheme: other mixing and evolution periods can be added to increase dimensions

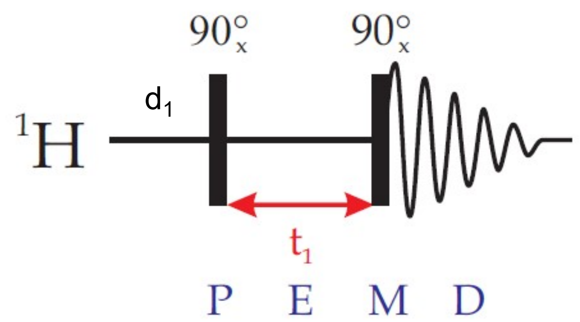


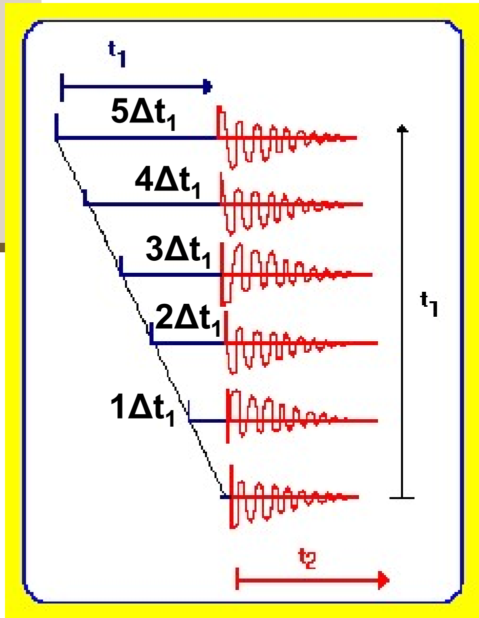
Example: COSY – mixing is scalar coupling

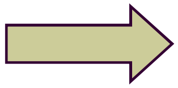


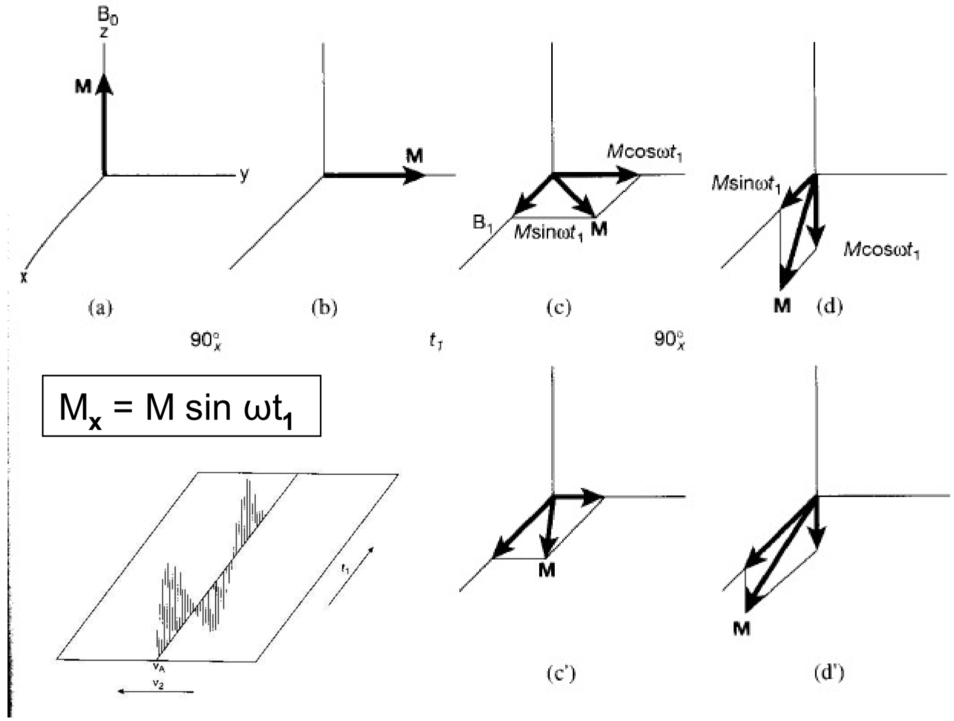
Introduction to ^1H-^1H COSY







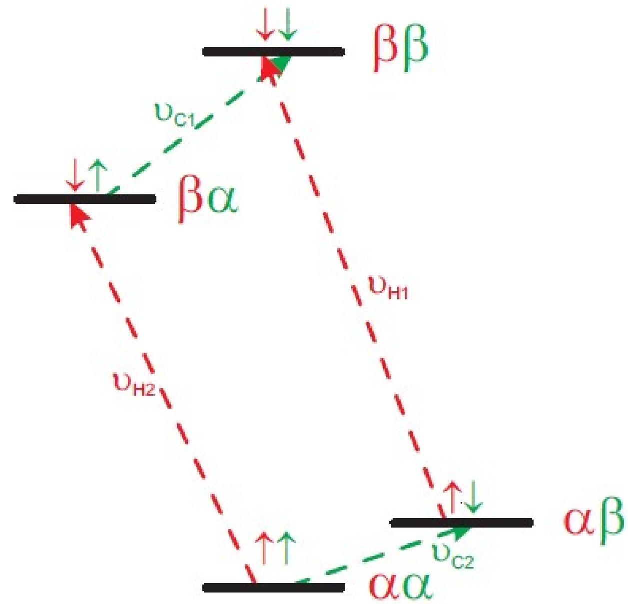


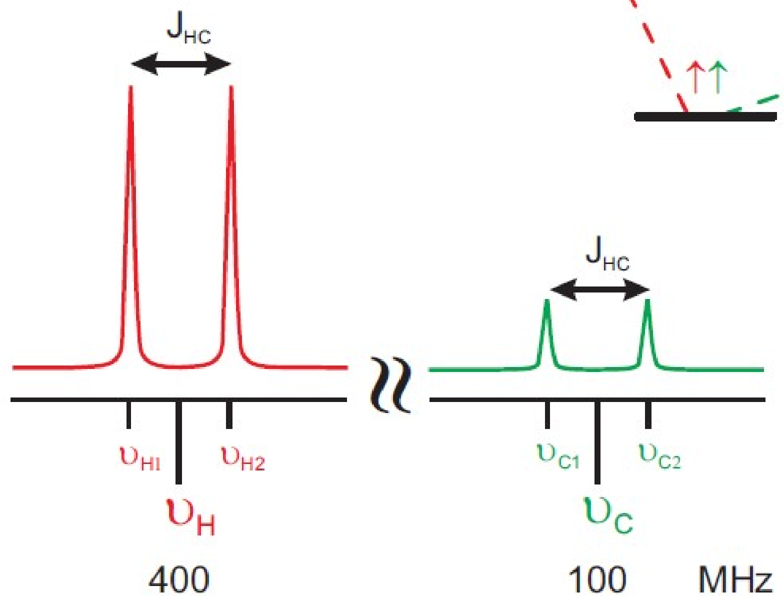


Introduction to polarization transfer for heteronuclear NMR experiments

Polarization Transfer

The fundamental basis of ***polarization transfer*** experiments exploits the greater Boltzmann population of a more sensitive nuclei (S) like ^{1}H and transfers it to an insensitive (I) nucleus like ^{13}C.



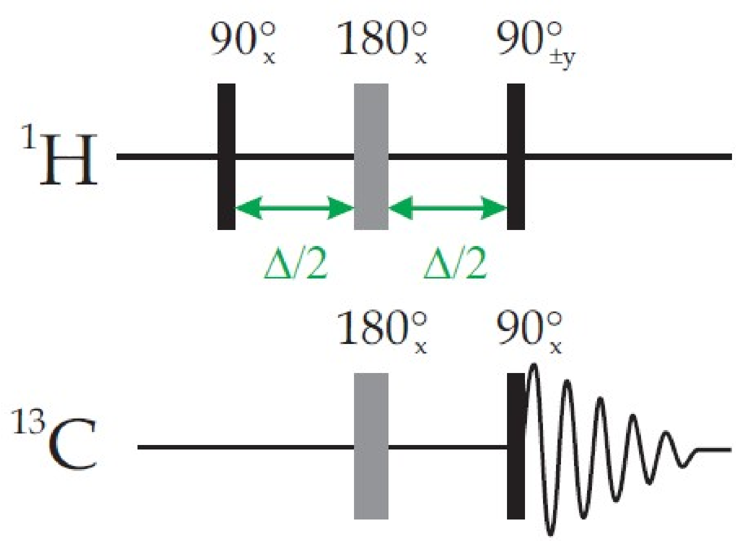


Introduction to <u>INEPT</u> by polarization transfer for heteronuclear NMR experiments

Insensitive Nuclei Enhancement by Polarization Transfer (INEPT).

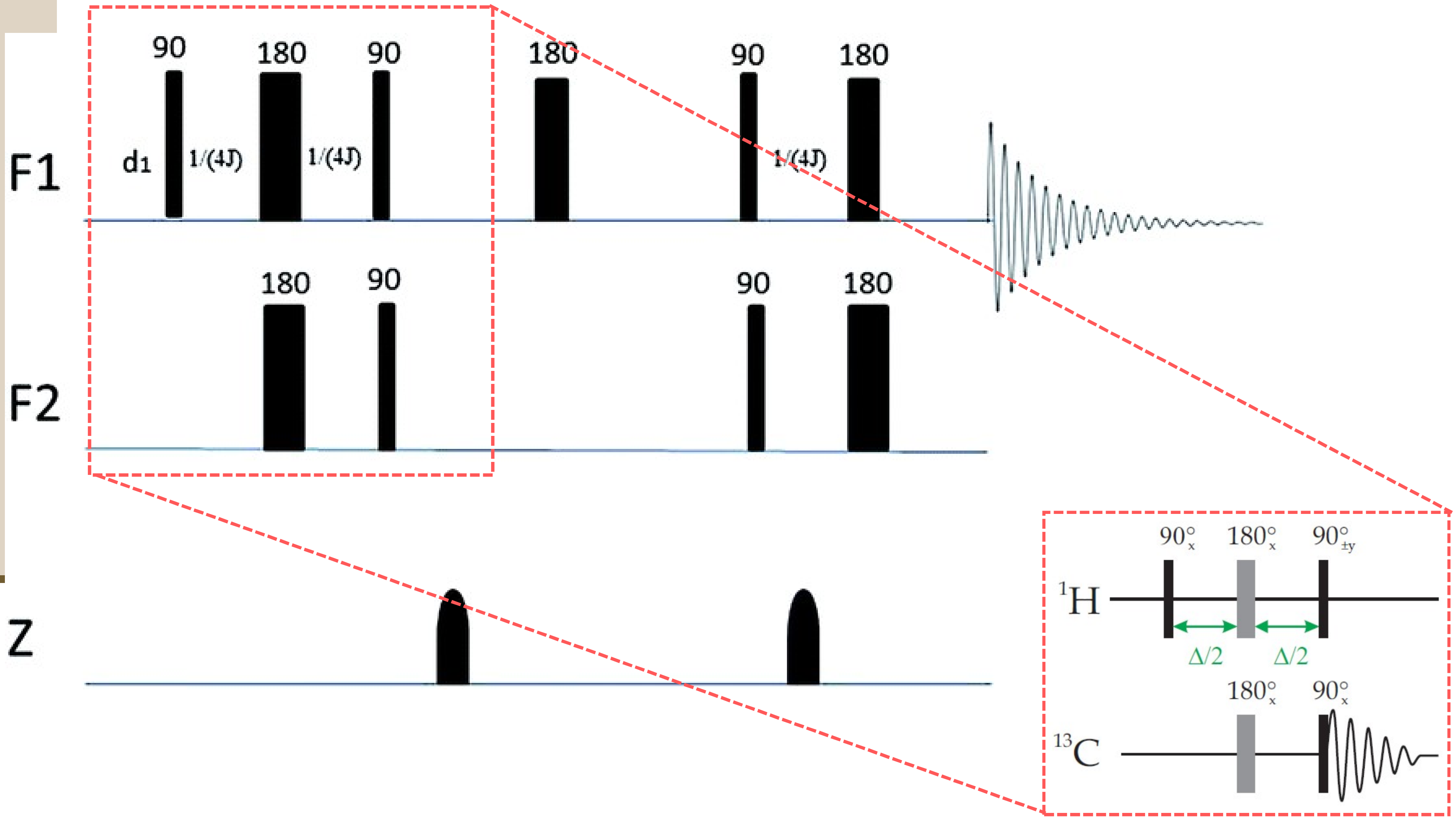
The INEPT sequence provides a way of inverting one half of all HC doublets regardless of chemical shift. It does this without the need for selective pulses.

The sequence is an adaptation of the spin echo sequence.

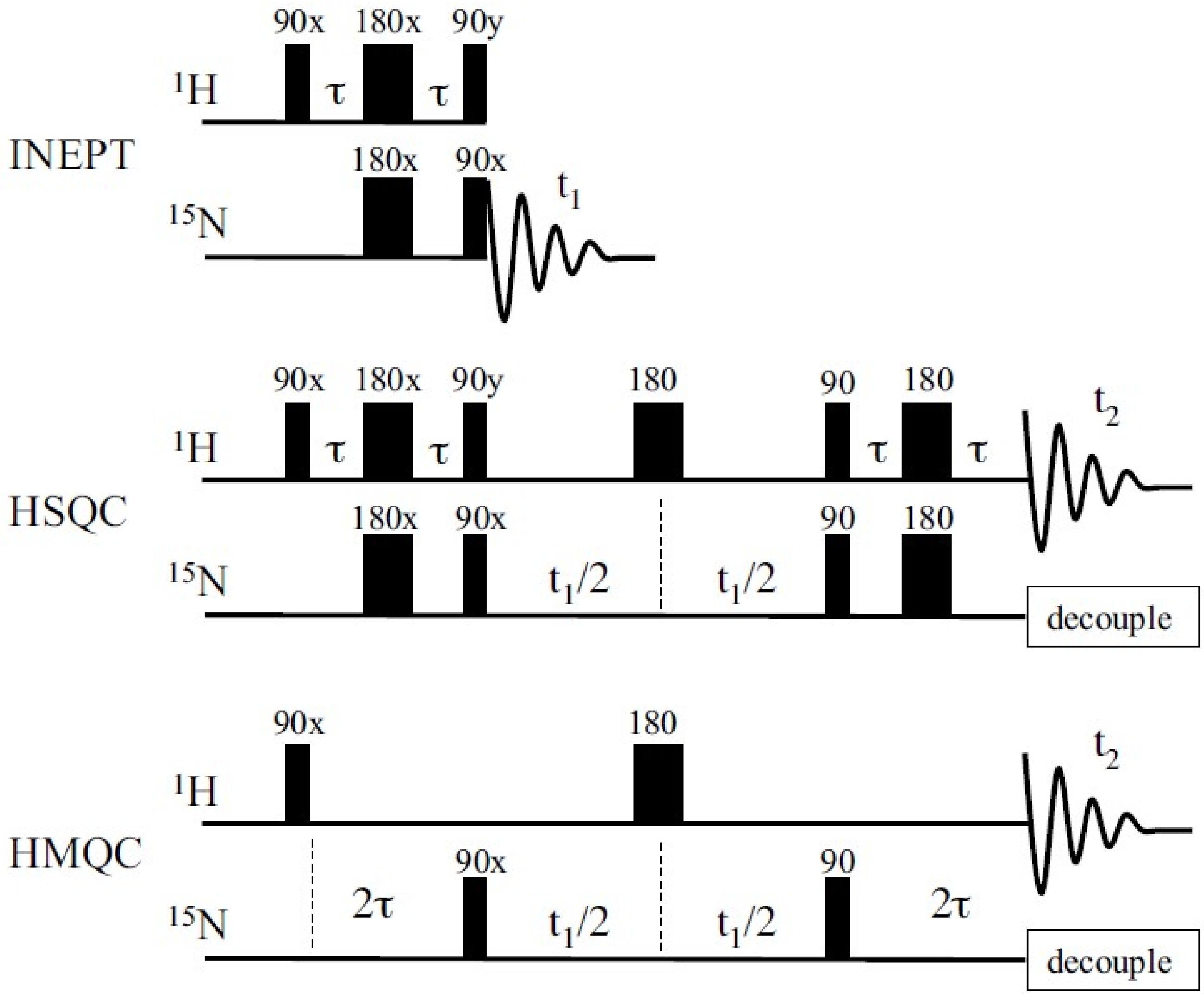


$\Delta/2 = 1/(4\ J)$

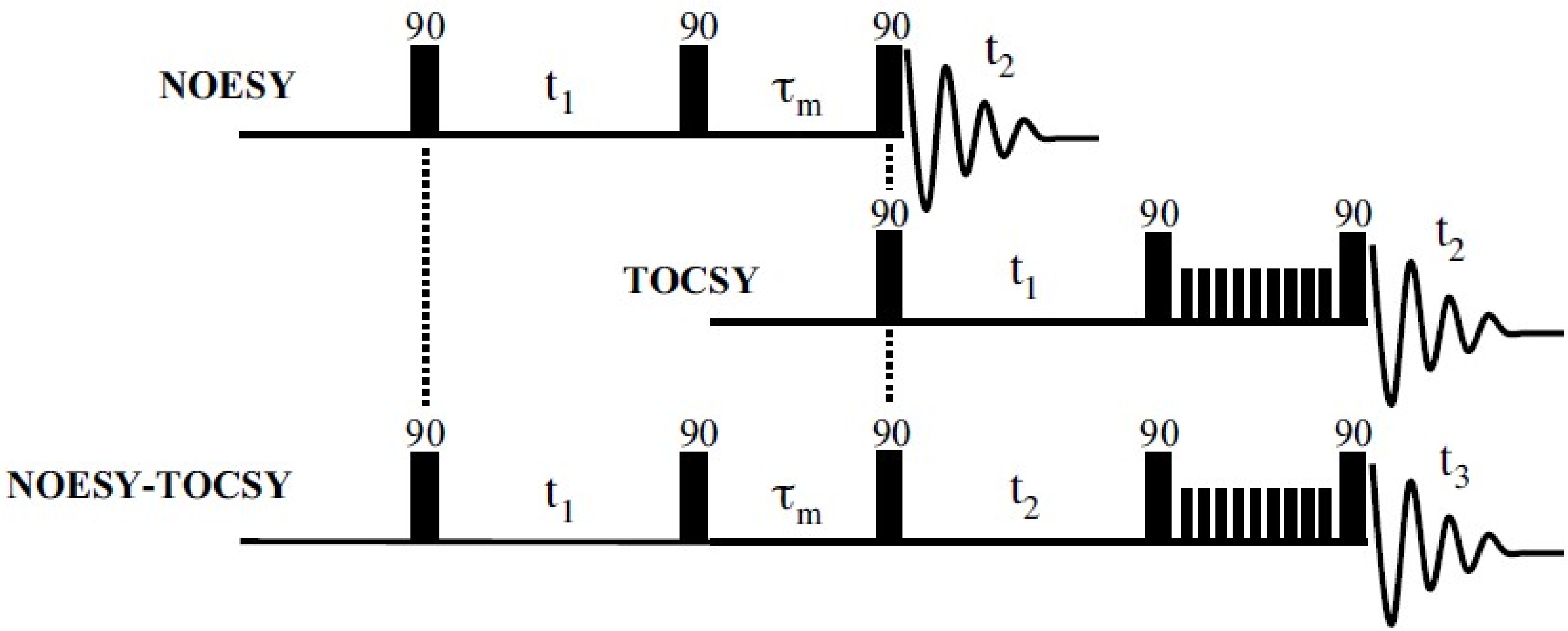
Pulse sequence of a gradient HSQC spectrum



INEPT, HSQC and HMQC pulse sequences

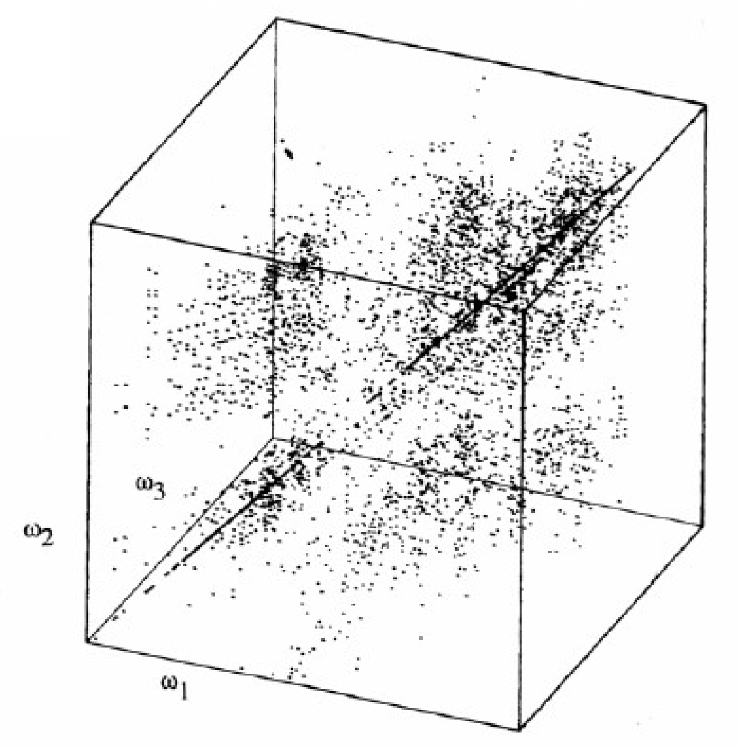


Example of a 3D ^1H-^1H NOESY-TOCSY experiment

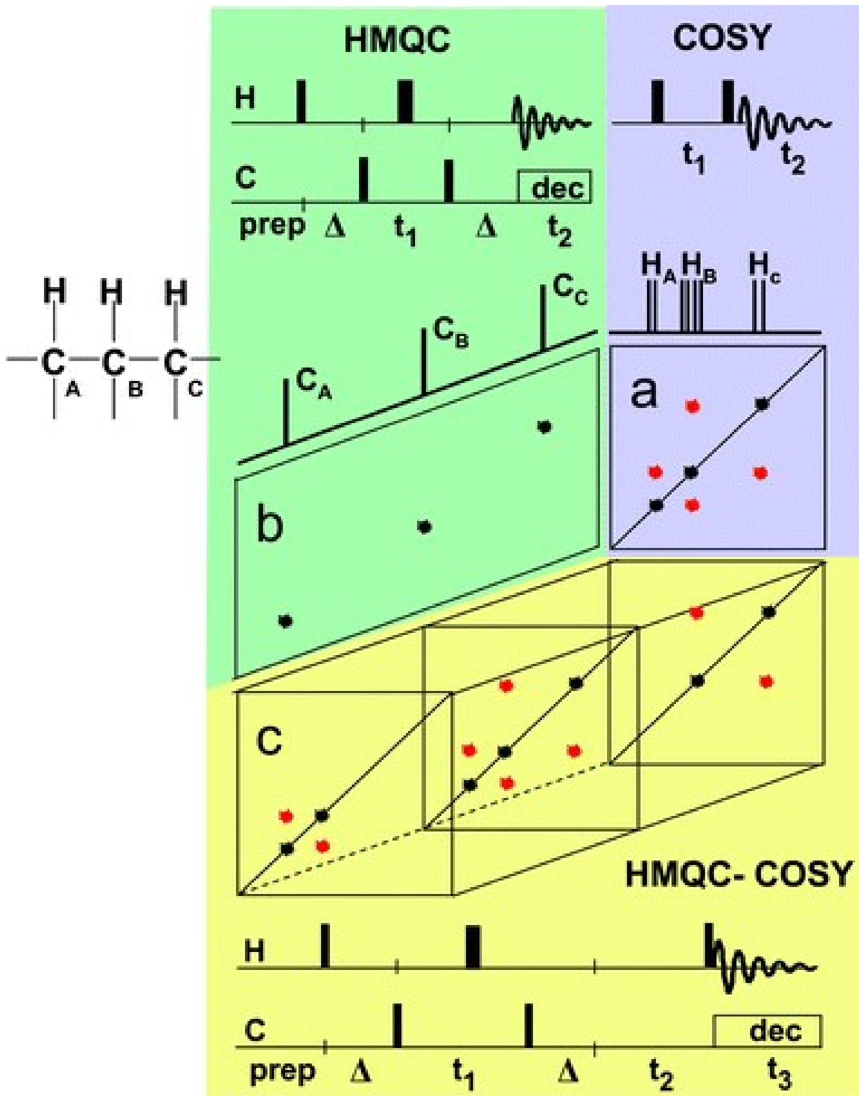


Example of a 3D ^1H-^1H NOESY-TOCSY experiment

- Example: 3D NOESY-TOCSY of parvalbumin (108 amino acids)
  - -8.7 mM
  - -170 hours (~ 7 days)
  - -50,000 cross peaks !



Example of a 3D ^1H-^{13}C HMQC-COSY experiment



Example of a 3D ^1H-^{13}C NOESY-HSQC experiment