

NMR Spectroscopy: Principles and Applications

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1D - Methods

Lecture 5

1D-NMR

To fully appreciate the workings of 1D NMR experiments we need to at least consider two coupled spins. Sometimes we need to go up to three coupled spins, particularly when we look at hetero nuclear spins such as Carbon-13 isotope. We will start from the simple one pulse NMR experiment in terms of the product operator approach and discuss various aspects of the pulse sequences.

Hamiltonian

Two Spins $I=1/2$ and J Coupling

Let us use the two spin Hamiltonian in which each spin with spin $I=1/2$ and J Coupling between them.

$$\hat{H} = \nu_{01}\hat{I}_{1z} + \nu_{02}\hat{I}_{2z} + J_{12}\hat{I}_{1z}\hat{I}_{2z} \quad \text{in Hz for the case } |\nu_{01} - \nu_{02}| \gg J_{12}$$

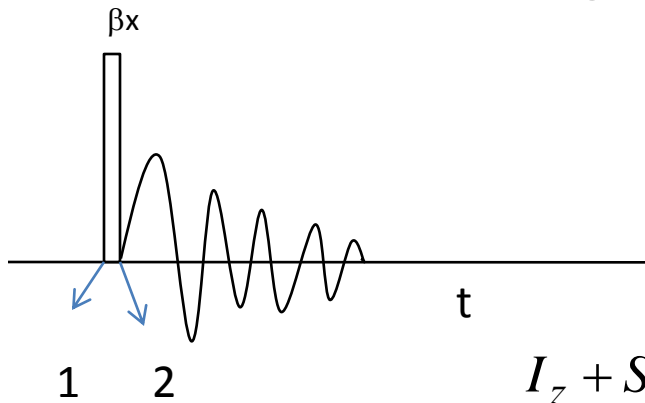
$$\hat{H} = \Omega_1\hat{I}_{1z} + \Omega_2\hat{I}_{2z} + 2\pi J_{12}\hat{I}_{1z}\hat{I}_{2z} \quad \text{in rotating frame and rad s}^{-1}$$

We can also write the equation with notation I and S for the two spins as

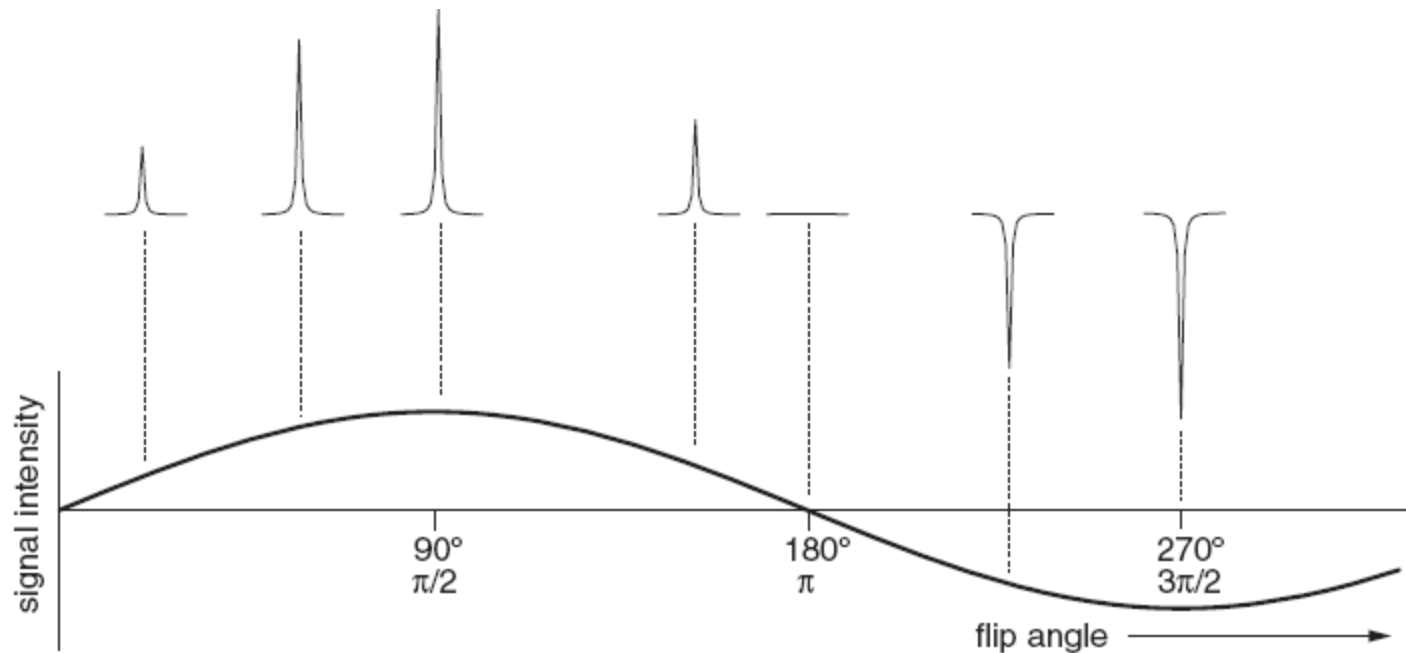
$$\hat{H} = \Omega_I\hat{I}_z + \Omega_S\hat{S}_z + 2\pi J_{IS}\hat{I}_z\hat{S}_z \quad \text{in rotating frame and rad s}^{-1}$$

In subsequent discussions we will drop the hat from the operators and represent them as normal face italic character for convenience.

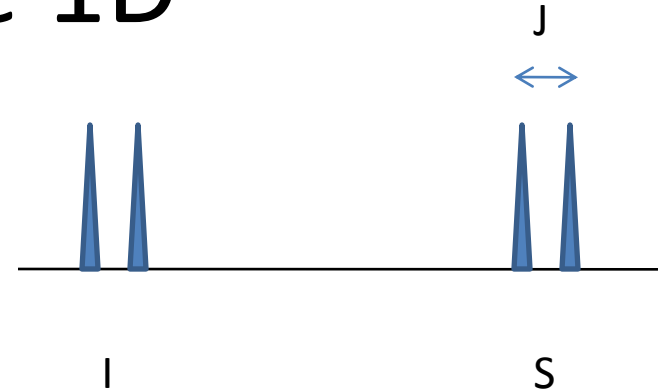
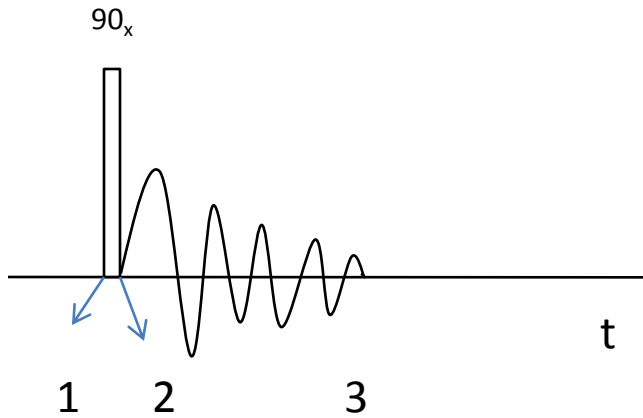
Pulse Calibration



$$I_z + S_z \xrightarrow[\xi]{\beta_0} I_z \cos \beta - I_y \sin \beta + S_z \cos \beta - S_y \sin \beta$$



Simple 1D

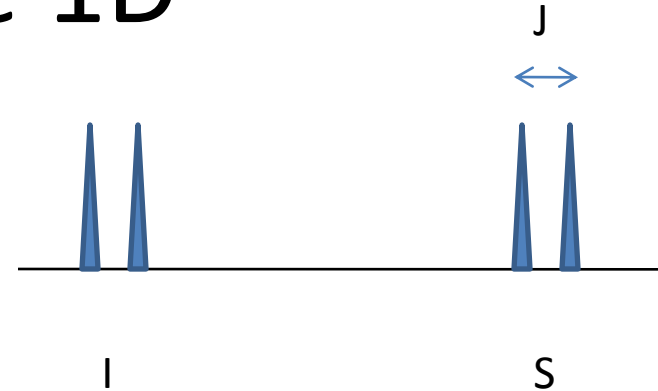
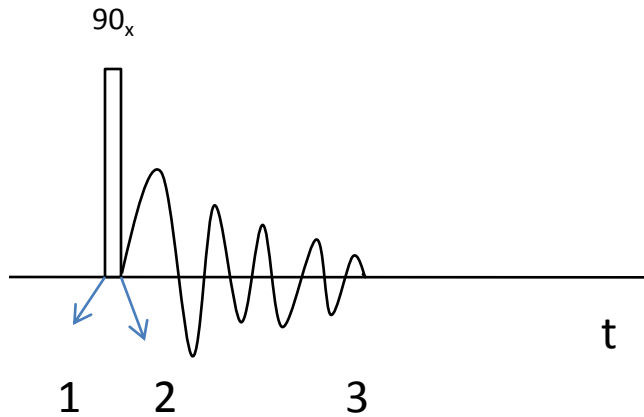


$$\begin{array}{l}
 1 \quad I_z + S_z \\
 \quad \downarrow 90_x \\
 2 \quad -I_y - S_y \\
 \quad \downarrow (\Omega_I I_z + \Omega_S S_z)t \\
 3 \quad -I_y \cos(\Omega_I t) + I_x \sin(\Omega_I t) \\
 \quad -S_y \cos(\Omega_S t) + S_x \sin(\Omega_S t)
 \end{array}$$

$$\begin{array}{l}
 -I_y \cos(\Omega_I t) \cos(\pi J_{IS} t) + 2I_x S_z \cos(\Omega_I t) \sin(\pi J_{IS} t) \\
 + I_x \sin(\Omega_I t) \cos(\pi J_{IS} t) + 2I_y S_z \sin(\Omega_I t) \sin(\pi J_{IS} t) \\
 - S_y \cos(\Omega_S t) \cos(\pi J_{IS} t) + 2S_x I_z \cos(\Omega_S t) \sin(\pi J_{IS} t) \\
 + S_x \sin(\Omega_S t) \cos(\pi J_{IS} t) + 2S_y I_z \sin(\Omega_S t) \sin(\pi J_{IS} t)
 \end{array}$$

$(\pi J_{IS} 2I_z S_z)t$

Simple 1D

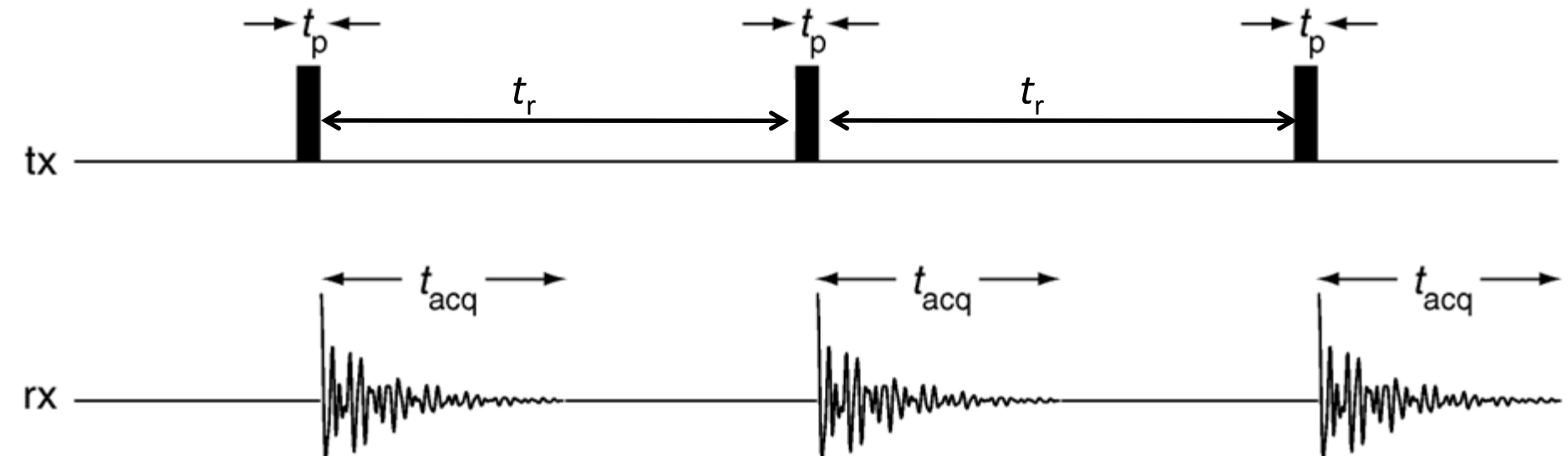


1 $I_z + S_z$
 $\downarrow 90_x$
 2 $-I_y - S_y$

As we know the physical properties of the operators we could have directly predicted the NMR spectrum from point 2 in the sequence. The operators at point 2 are in-phase y magnetization of spins I and S and thus the spectrum should be a in-phase doublet with splitting J at frequencies Ω_I and Ω_S . Also it is enough to follow one spin through the pulse sequence.

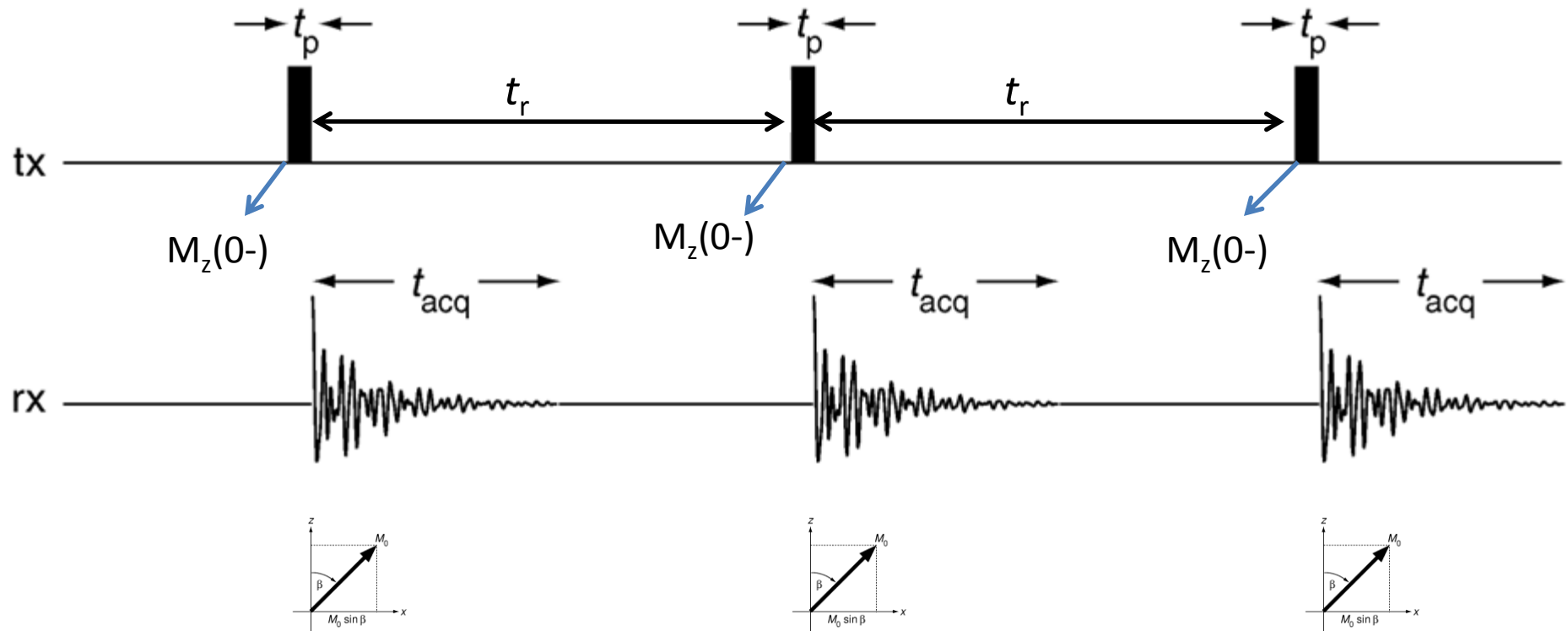
Optimum Pulse Flip Angle

It is not optimum to use $\pi/2$ flip angle for the pulse as the experiment is repeated several times for signal averaging and then one should wait more than 5 times the longitudinal relaxation time to build up the equilibrium magnetization.



Optimum Pulse Flip Angle

If we assume that the transverse magnetization is completely decayed by the time the next signal averaging pulse is applied, we can calculate the steady state z-magnetization and the corresponding transverse magnetization can be calculated.

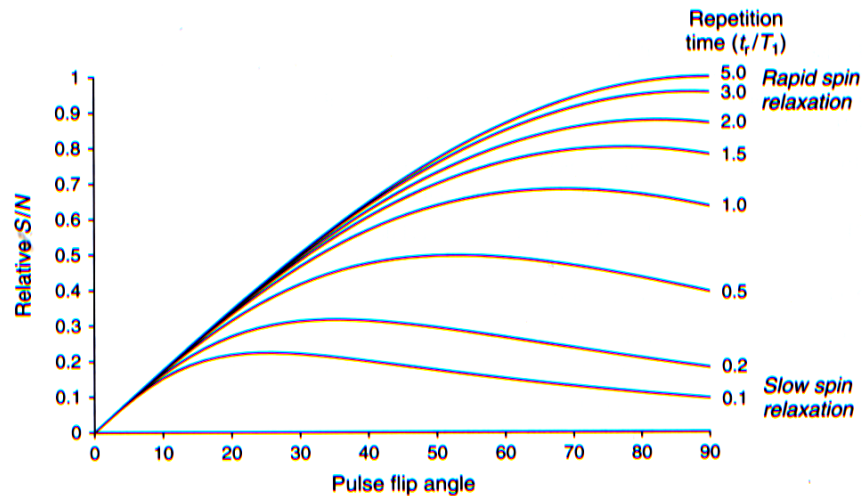


Optimum Pulse Flip Angle

The detected signal can be deduced to be

$$M_x(0_+) = M_0 \frac{1 - \exp\left(-\frac{t_r}{T_1}\right)}{1 - \exp\left(-\frac{t_r}{T_1}\right) \cos \beta} \sin \beta$$

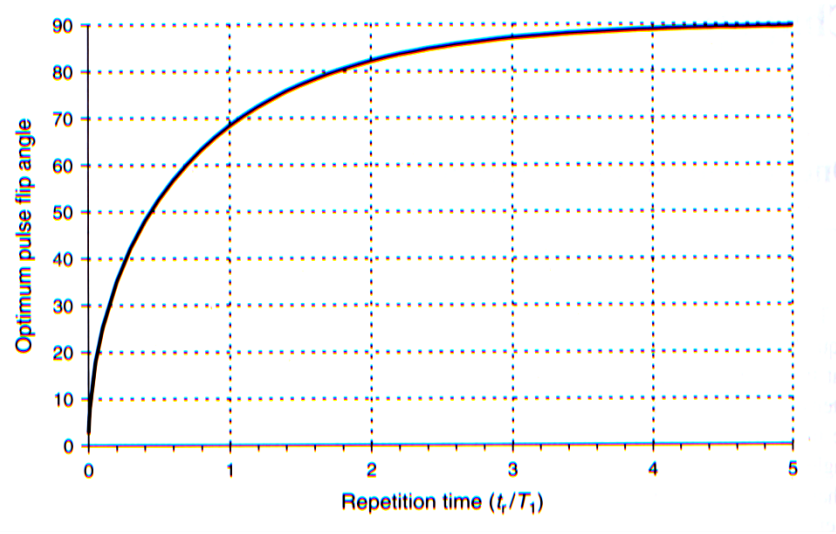
And the signal intensity as relative S/N plotted for various (t_r / T_1) as a function of the flip angle is given below.



Optimum Pulse Flip Angle

The maximum signal is not at $\beta = \pi/2$, the optimum flip angle, known as **Ernst angle**, β is given by

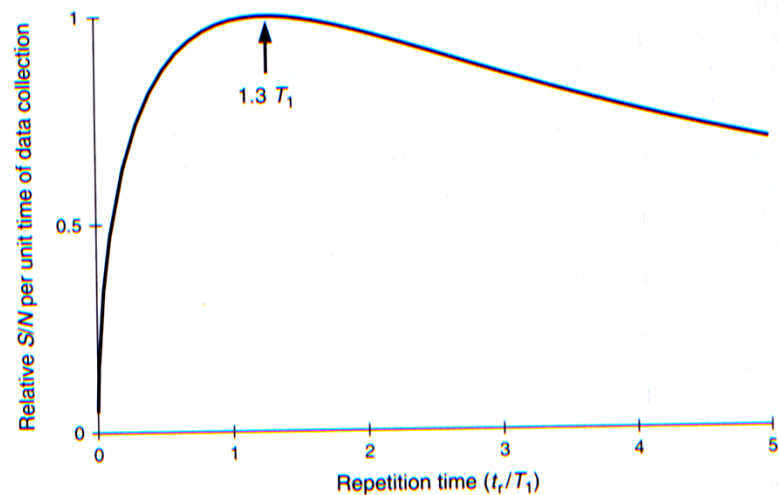
$$\cos \beta_{opt} = \exp(-t_r / T_1)$$



For shorter recycle time β_{opt} decreases. For $t_r \sim T_1$, $\beta_{opt} \sim 68^\circ$.

Optimum Recycle Delay

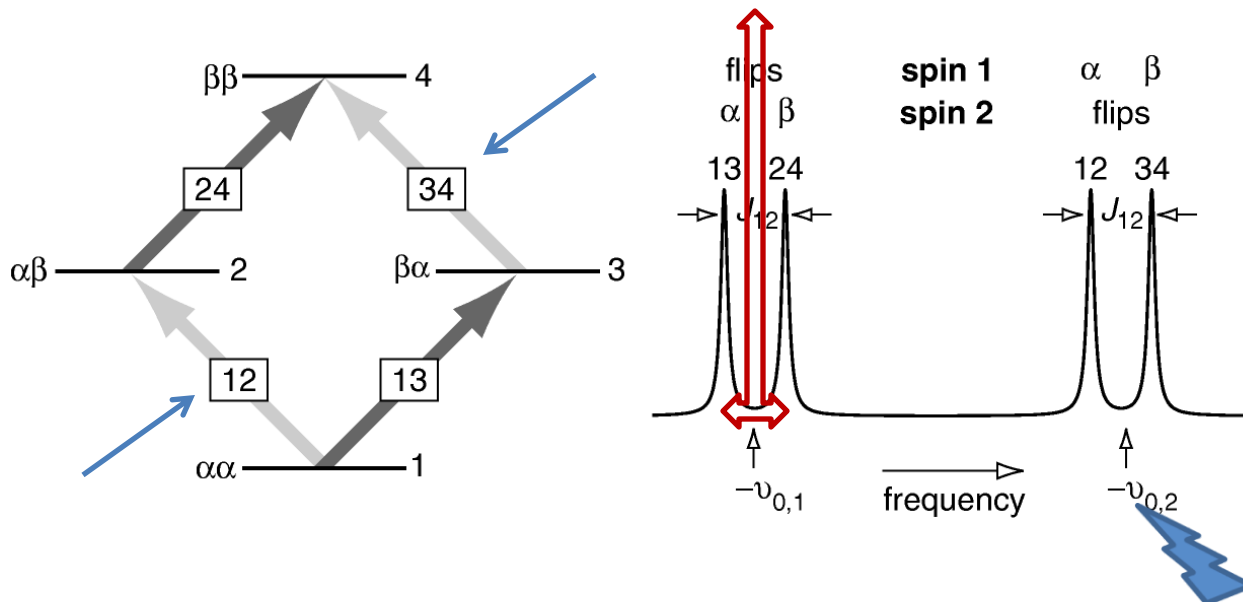
The optimum recycle delay for a pulse angle of $\beta = \pi/2$ can also be plotted as a function of the ratio of t_r/T_1



And the delay $t_r \sim 1.3 T_1$ is optimum.

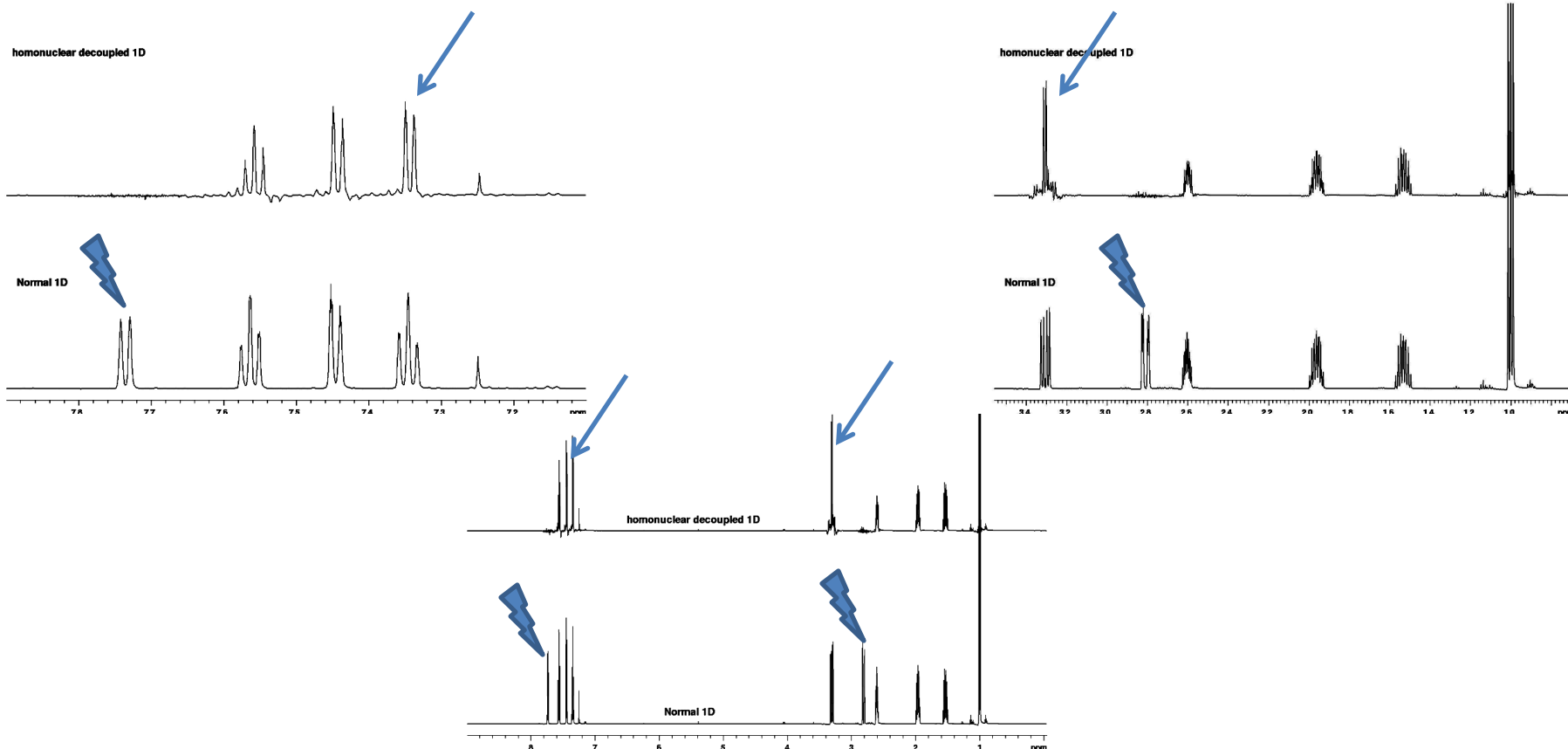
1D with Decoupling

The one pulse experiment gives a doublet for each spins when there is a J -coupling between them. Spin 1 multiplet frequencies depend on the state of spin 2 in α or β state. If selective RF irradiation (applied during detection) rapidly changes mixes the α and β state of spin 2 the spin multiplet will collapse and this process is called decoupling.



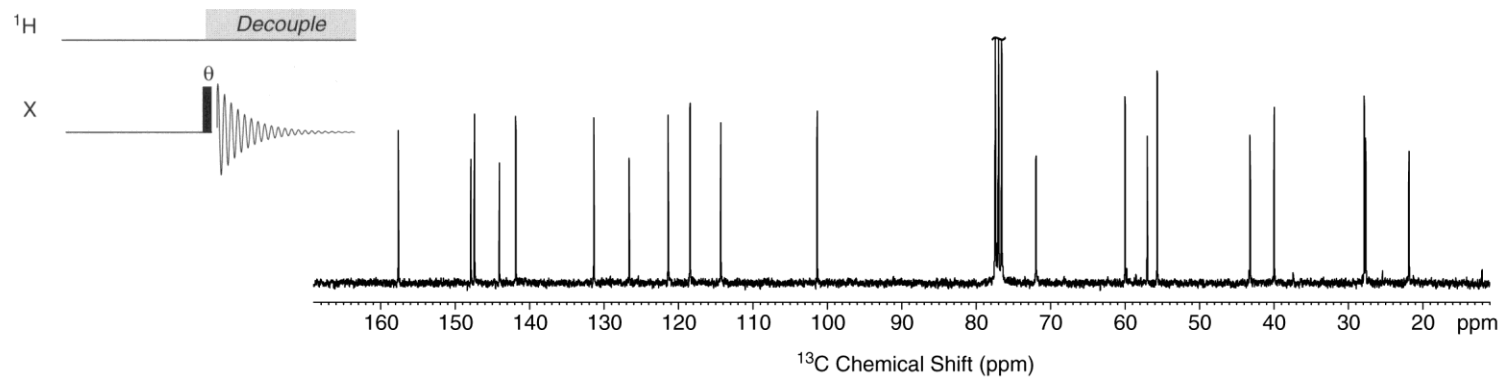
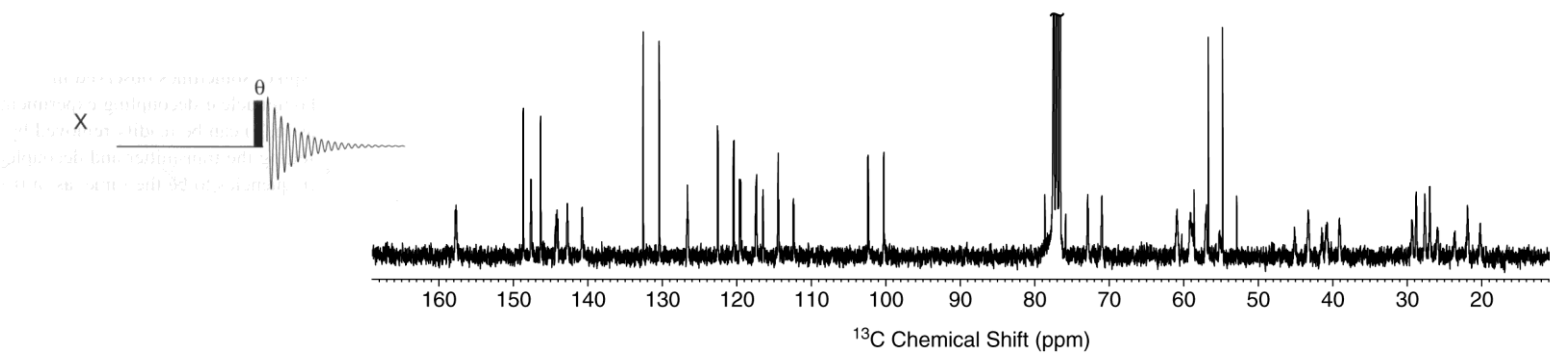
1D with Decoupling

^1H – spectra with decoupling (homonuclear decoupling).



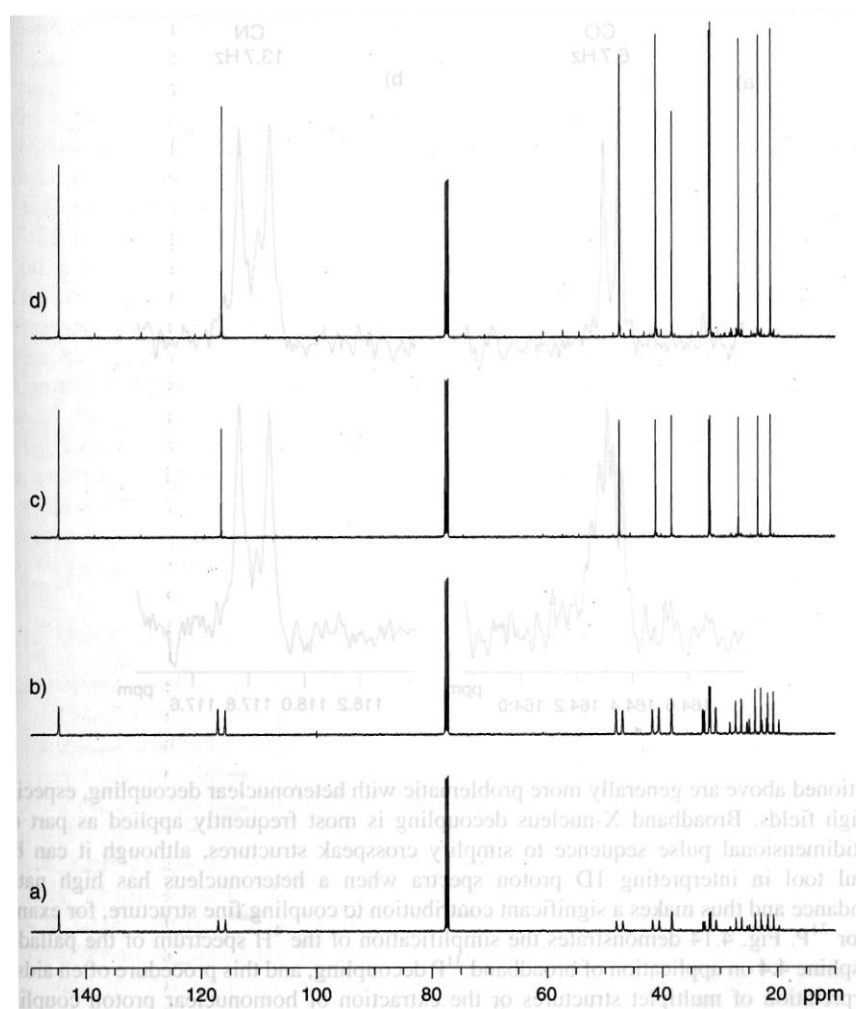
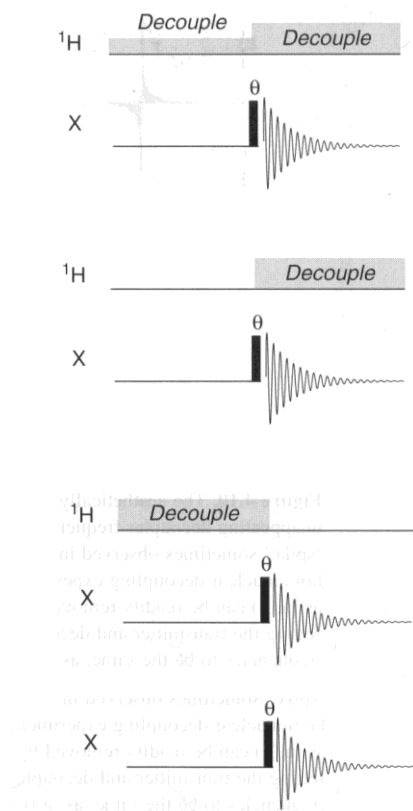
1D with Decoupling

Decoupling is particularly useful in recording ^{13}C spectrum.

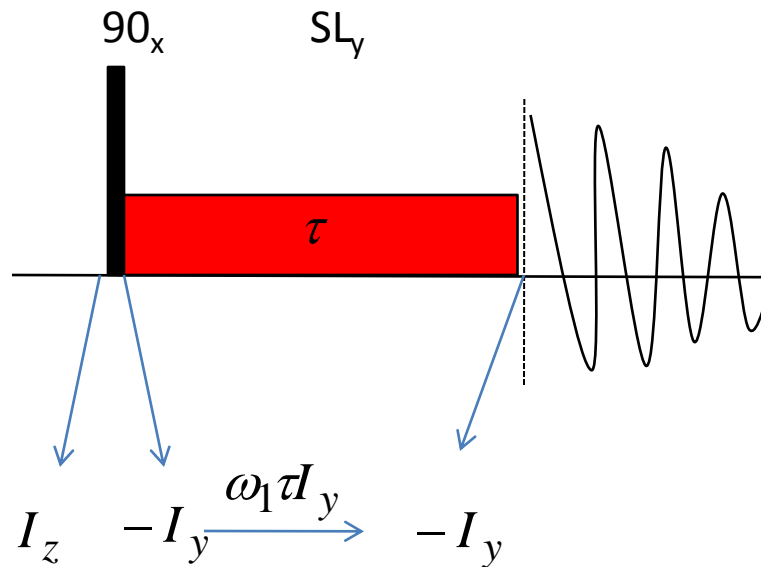


1D with Decoupling

There are variants of the basic experiment to enhance the ^{13}C spectrum.

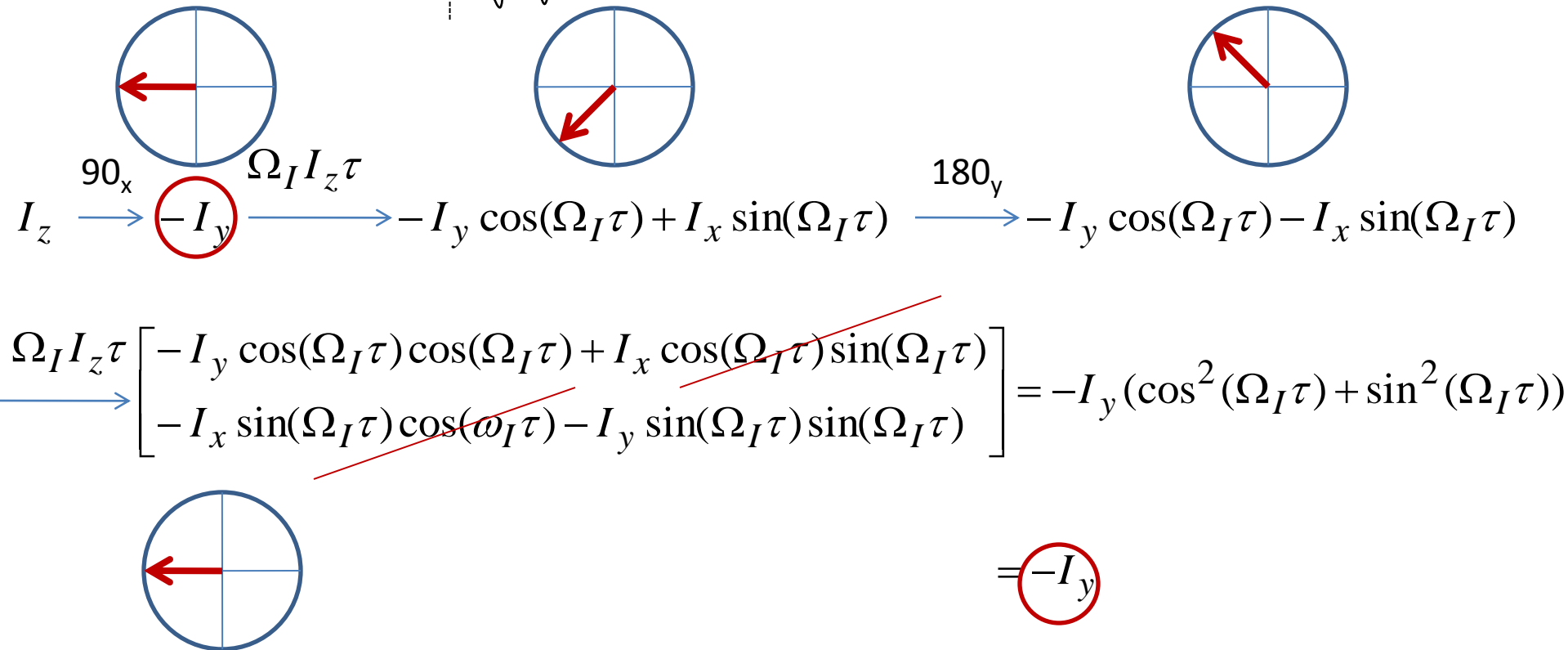
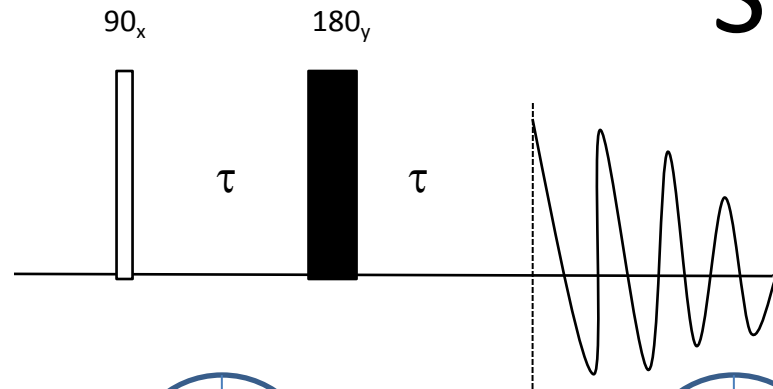


Spin Lock



During the delay t the I_y coherence of the spins do not evolve and is locked along the y -axis along which the RF is applied. This process called spin locking.

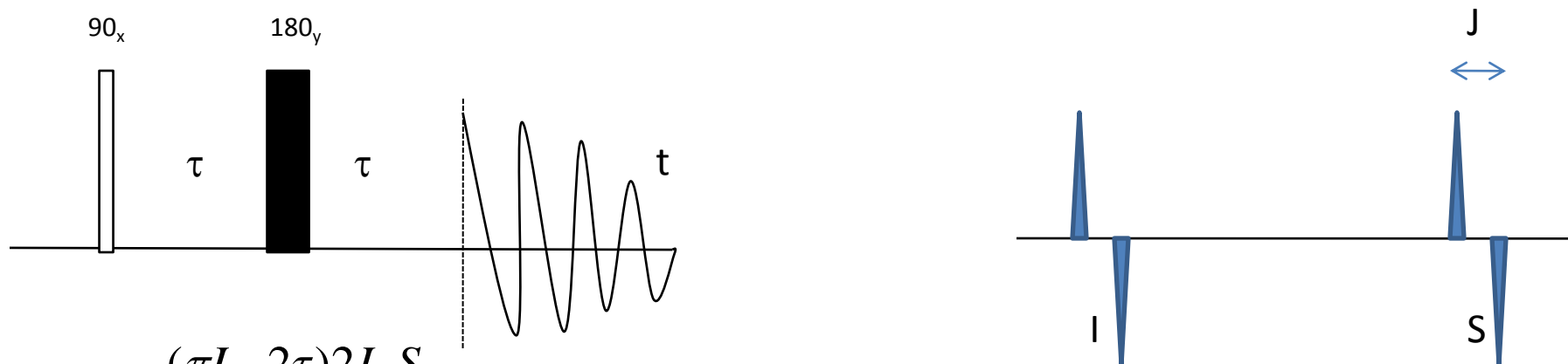
Spin Echo



At the end of 2τ period chemical shift evolution is refocused.

Spin Echo –J Modulation

Using the knowledge that shift evolution is refocused, the J evolution can be calculated.



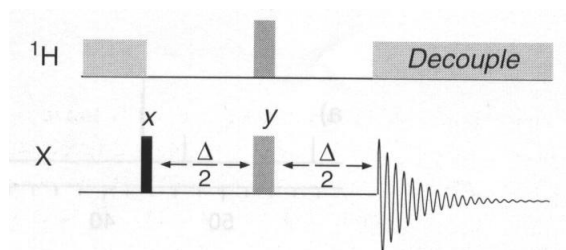
$$-I_y \xrightarrow{(\pi J_{IS} 2\tau) 2I_z S_z} -I_y \cos(\pi J_{IS} 2\tau) + 2I_x S_z \sin(\pi J_{IS} 2\tau)$$

$$\tau = \frac{1}{4J} : 2I_x S_z \xrightarrow{\text{FID}} \begin{aligned} &2I_x S_z \cos(\Omega_I t) \cos(\pi J t) + I_y \cos(\Omega_I t) \sin(\pi J t) + \\ &2I_y S_z \sin(\Omega_I t) \cos(\pi J t) - I_x \sin(\Omega_I t) \sin(\pi J t) \end{aligned}$$

At the end of 2τ period the anti-phase coherence is generated.

Spin Echo –J Modulation –Spectrum Editing

The dependence of echo on J coupling can be used to edit carbon spectrum to identify C, CH, CH₂, CH₃ type carbons.



$$\text{CH:} \quad -S_y \xrightarrow{(\pi J_{IS}\Delta)2I_z S_z} -S_y \cos(\pi J_{IS}\Delta) + 2S_x I_z \sin(\pi J_{IS}\Delta)$$

$$\boxed{-S_y \cos \theta} + 2S_x I_z \sin \theta \quad \text{with } \pi J_{IS}\Delta = \theta$$

$$\downarrow (\pi J_{IS}\Delta)2K_z S_z$$

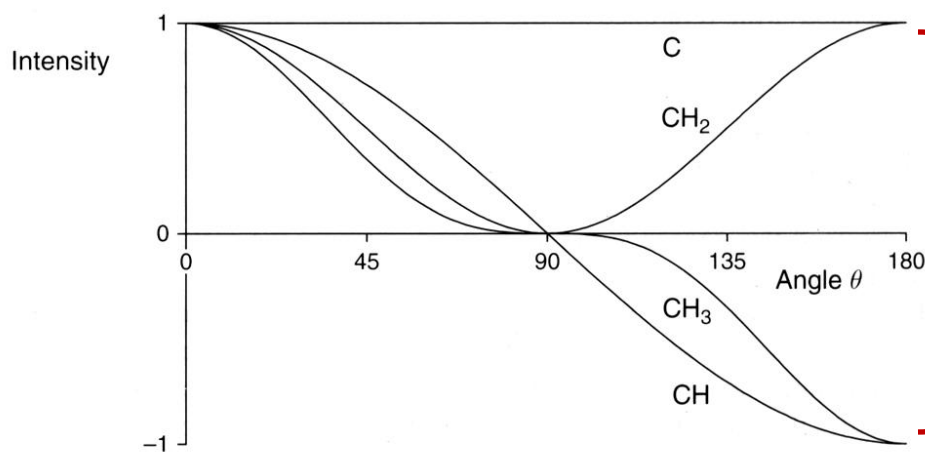
$$\text{CH}_2: \quad \boxed{-S_y \cos^2 \theta} + 2S_x K_z \sin \theta \cos \theta + 2S_x I_z \sin \theta \cos \theta + 4S_y I_z K_z \sin^2 \theta \cos \theta$$

$$\downarrow (\pi J_{IS}\Delta)2L_z S_z$$

$$\text{CH}_3: \quad \boxed{-S_y \cos^3 \theta} + 2S_x L_z \sin \theta \cos \theta + \dots$$

Spin Echo –J Modulation –Spectrum Editing

The functional dependence can be used to identify the protonation of carbon. For $\theta = \pi$, C and CH_2 amplitudes are opposite of CH and CH_3 carbons.



$$\Delta = \frac{1}{J_{IS}} : \cos(\pi J_{IS} \Delta) = -1$$

$$\theta = \pi \text{ or } 180^\circ$$

$$C : 1$$

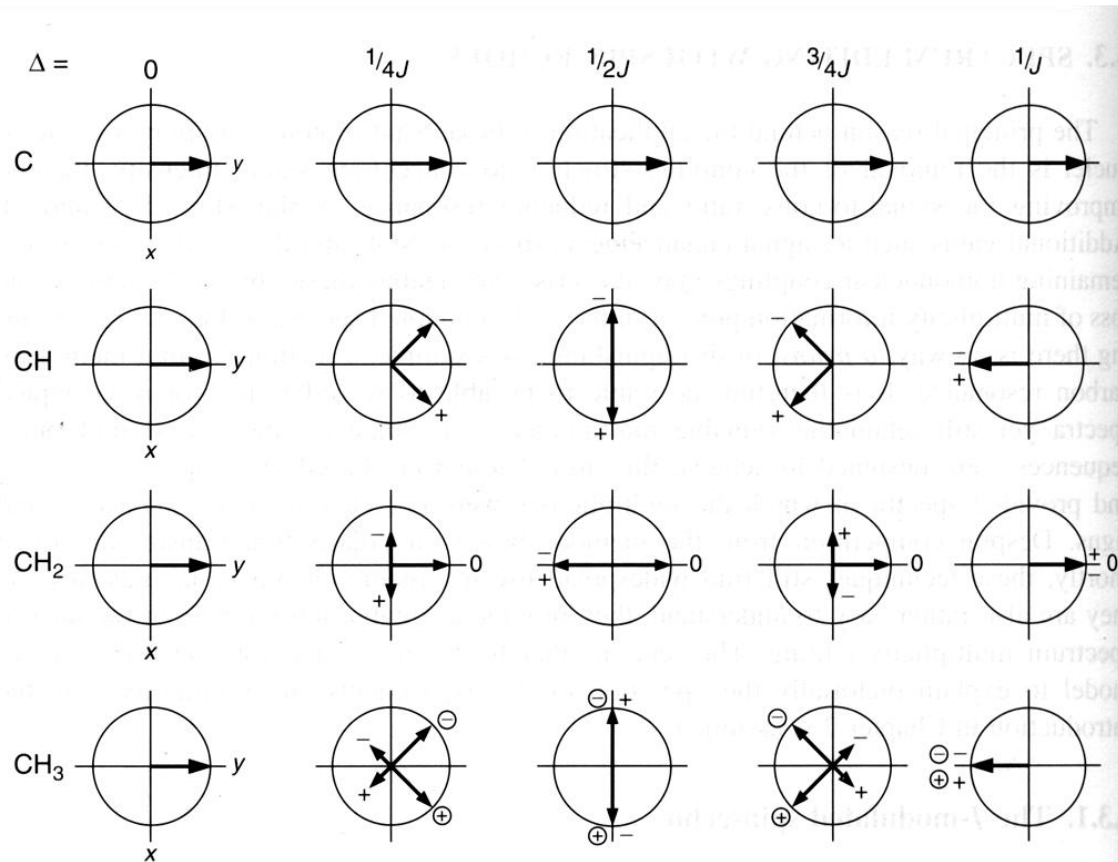
$$CH : \cos \theta$$

$$CH_2 : \cos^2 \theta$$

$$CH_3 : \cos^3 \theta$$

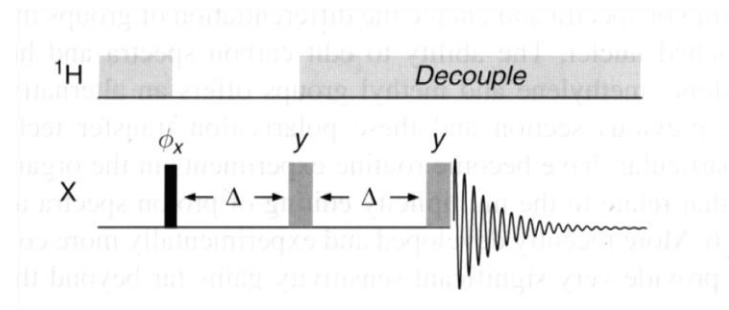
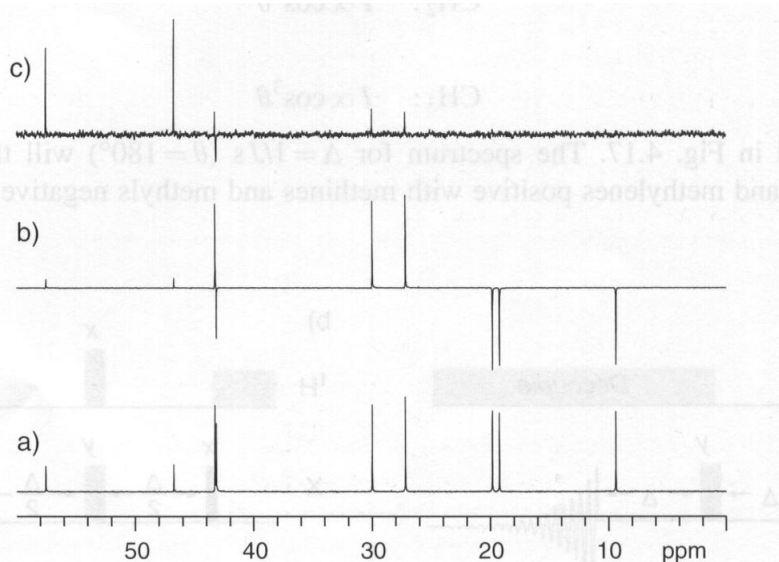
Spin Echo –J Modulation –Spectrum Editing

Each multiplet in the carbon spectrum of the spin system may be represented by a vector, then the evolution can be pictorially represented.



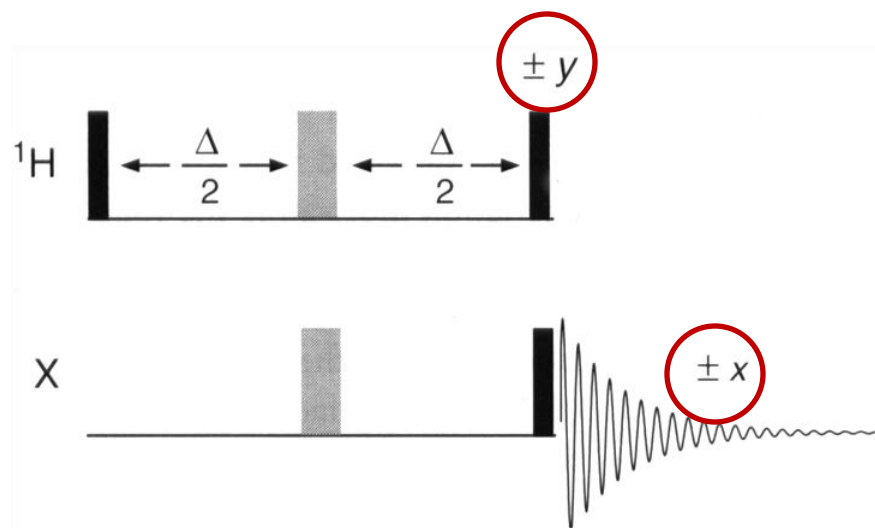
APT-Attached Proton Test

The APT experiment uses the functional dependence of θ and edits the carbon spectrum (a) Normal carbon 1D, (b) edited spectrum with $\theta = \pi$, and (c) $\theta = \pi/2$ only the quaternary carbon is selected. The first carbon pulse is not a $\pi/2$ pulse to optimize S/N with fast repetition times.

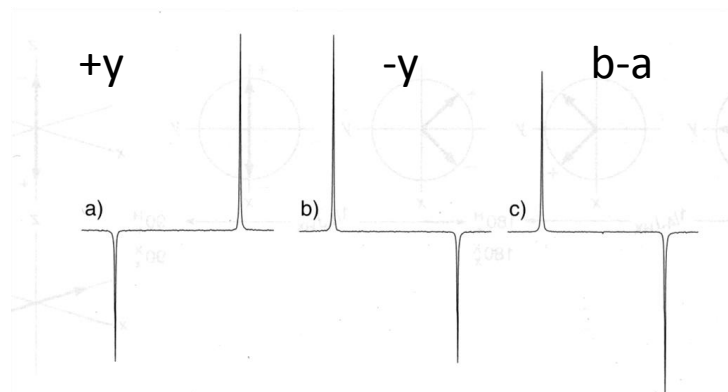
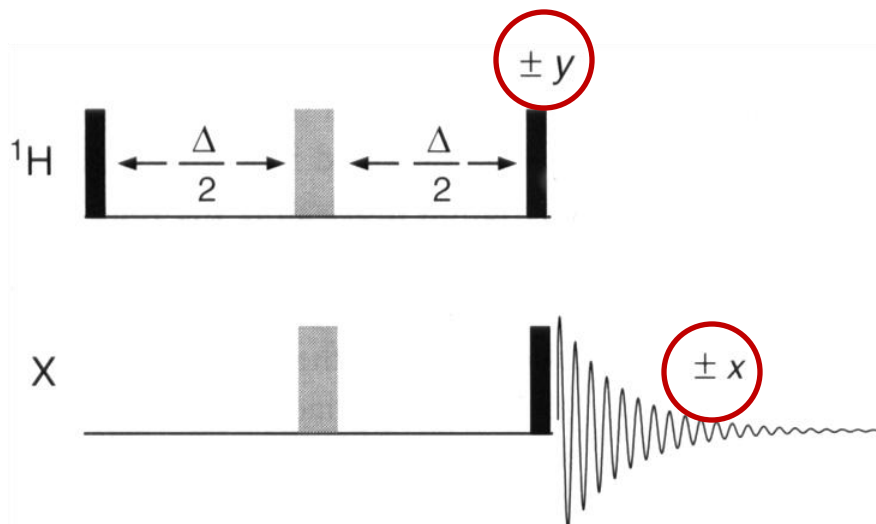


Polarization Transfer

There is a class of experiments that are called polarization transfer experiments and they transfer magnetization of abundant high γ nuclei magnetization to low abundant low γ nuclei – such as proton to carbon. One such type of experiment is called INEPT (Insensitive Nuclear Enhancement by Polarization Transfer).



INEPT



$$-\gamma_I I_y \xrightarrow{\pi I_x, \pi S_x, (\pi J_{IS} \Delta) 2 I_z S_z} \gamma_I I_y \cos(\pi J_{IS} \Delta) - \gamma_I 2 I_x S_z \sin(\pi J_{IS} \Delta)$$

$\downarrow \quad \pm \frac{\pi}{2} I_y, \pm \frac{\pi}{2} S_y$

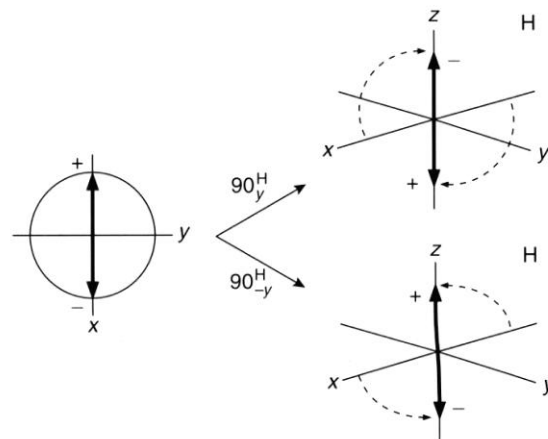
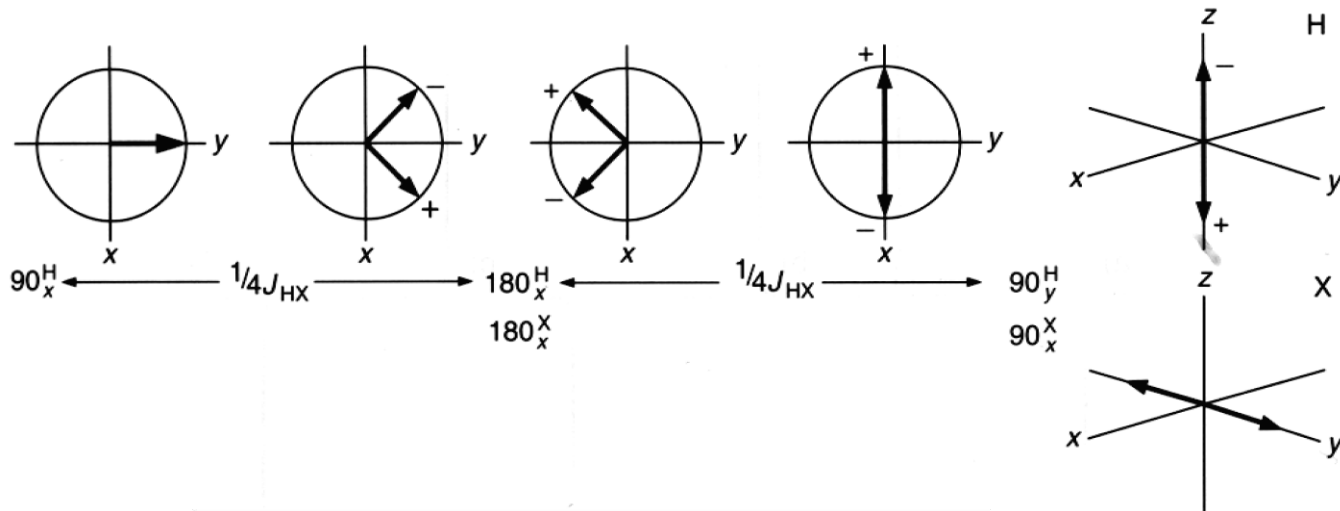
$$\gamma_I I_y \cos(\pi J_{IS} \Delta) \pm \gamma_I 2 I_z S_x \sin(\pi J_{IS} \Delta)$$

$$\pm \gamma_I 2 I_z S_x \sin(\pi J_{IS} \Delta) \xrightarrow{FID} \pm \gamma_I S_y \cos(\Omega_s t) \sin(\pi J_{IS} t) \text{ with } \Delta = \frac{1}{2 J_{IS}}$$

If $I = {}^1\text{H}$ and $S = {}^{13}\text{C}$ then $\gamma_I/\gamma_S = 4$, we have 4 times more signal than normal 1D.

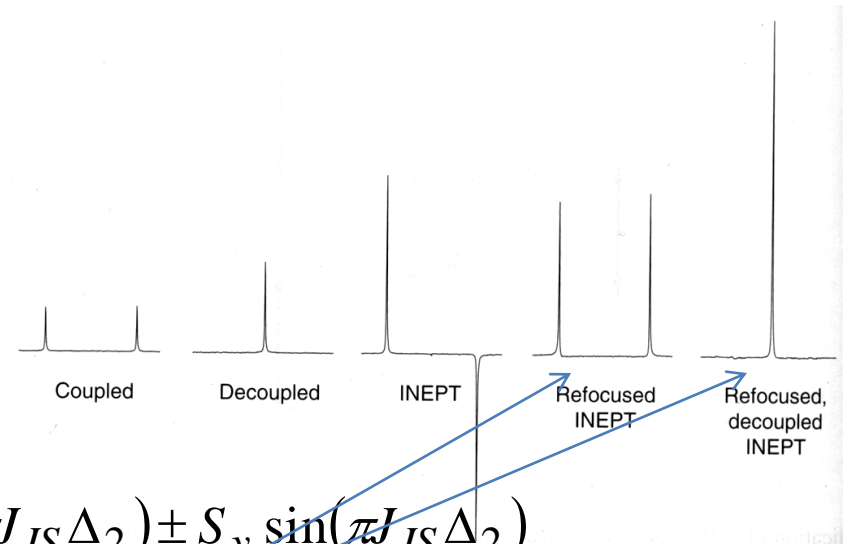
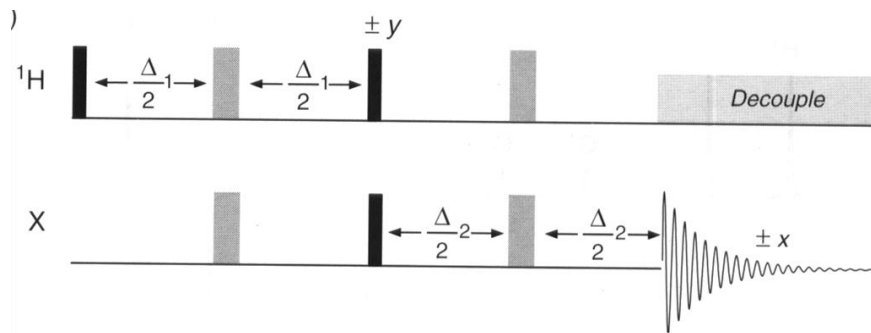
INEPT

One can also illustrate the evolution pictorially.



Refocused INEPT

The INEPT sequence can be extended to give in-phase multiplet so that decoupling can be performed. Such a pulse sequence is called refocused INEPT.



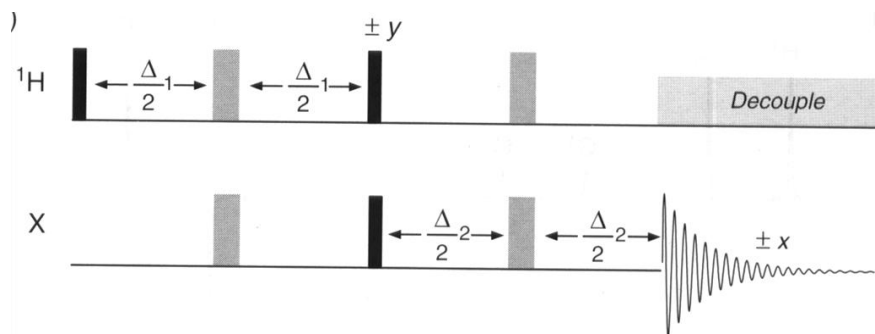
$$\pm \gamma_I 2I_z S_x \xrightarrow{\pi J_{IS} \Delta_2 2I_z S_z} \pm 2I_z S_x \cos(\pi J_{IS} \Delta_2) \pm S_y \sin(\pi J_{IS} \Delta_2)$$

$$\text{with } \pi J_{IS} \Delta_2 = \theta$$

$$\pm \gamma_I 2I_z S_x \xrightarrow{\pi J_{IS} \Delta_2 2I_z S_z} \pm 2I_z S_x \cos \theta \pm S_y \sin \theta$$

Editing with Refocused INEPT

The refocused INEPT can also be used to edit carbon spectrum as we did with APT experiment.

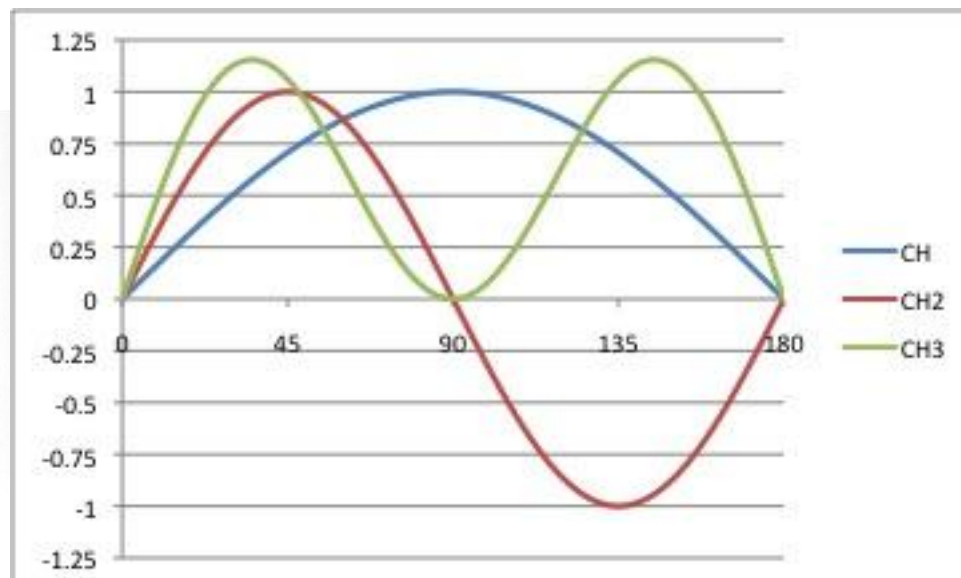
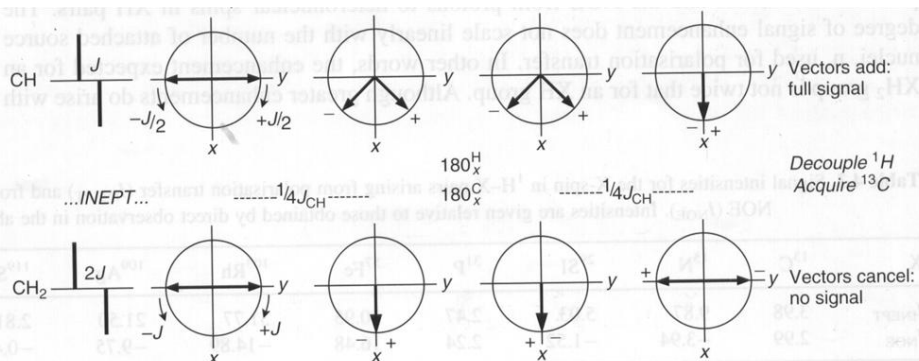


with $\theta = \pi J \Delta_2$

$\text{CH} : \sin \theta$

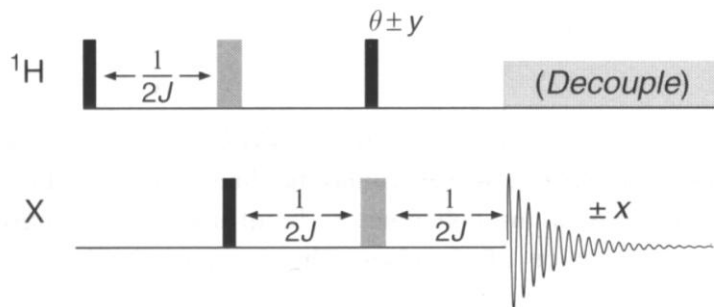
$\text{CH}_2 : 2 \sin \theta \cos \theta$

$\text{CH}_3 : 3 \sin \theta \cos^2 \theta$

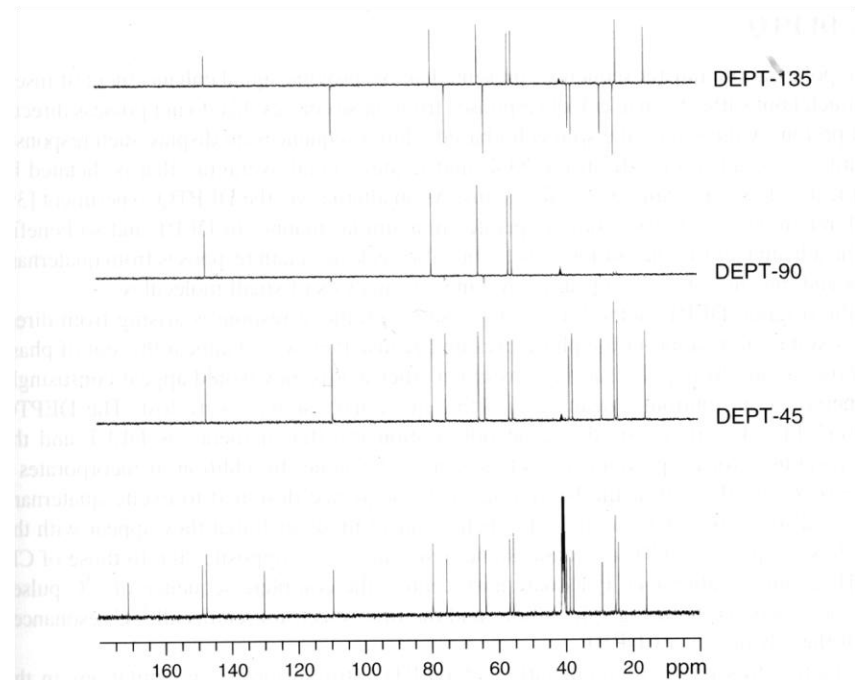
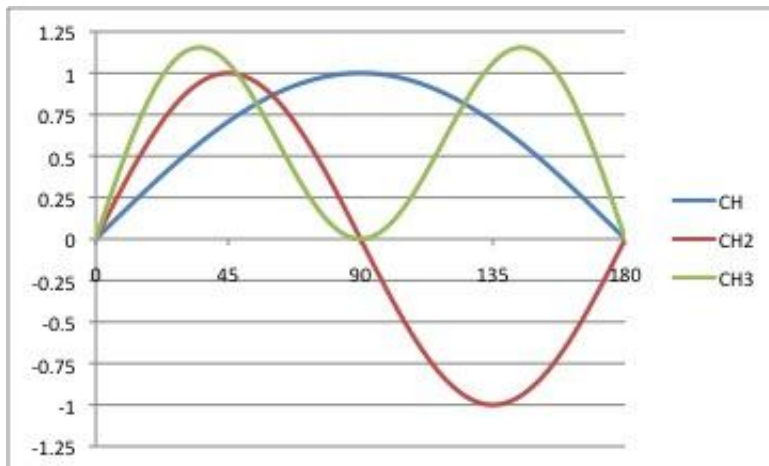


Editing with DEPT

To Edit using the refocused INEPT one has to vary the delay to get the desired θ . Instead, we modify the pulse sequence to achieve the same effect. The flip angle of ^1H pulse θ is varied to achieve the same result.



$$\text{CH} : \sin \theta; \text{CH}_2 : 2 \sin \theta \cos \theta; \text{CH}_3 : 3 \sin \theta \cos^2 \theta$$



Editing with DEPT

A suitable linear combination of the four spectra on the left yields the edited spectra on the right.

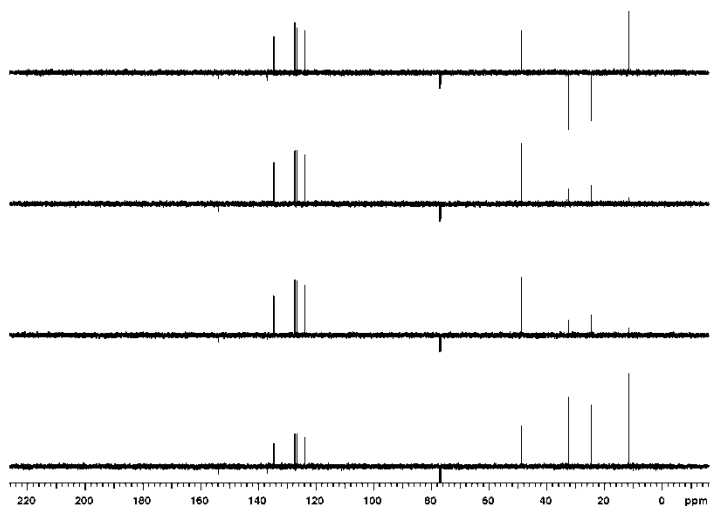
$$0.707CH + 1.0CH_2 + 1.061 CH_3$$

DEPT-135°

DEPT-90°

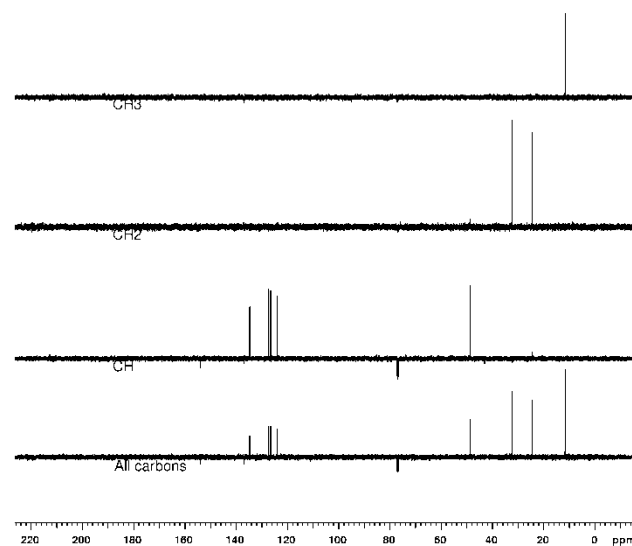
DEPT-90°

DEPT-45°



CH

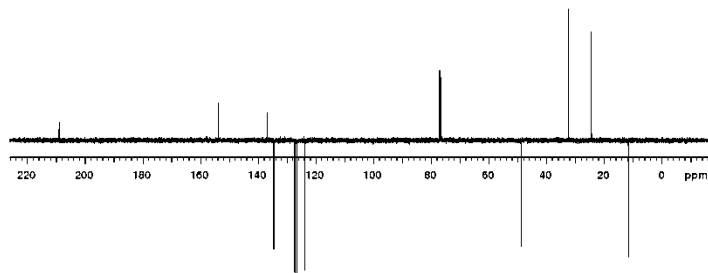
$$0.707CH + 1.0CH_2 + 1.061 CH_3$$



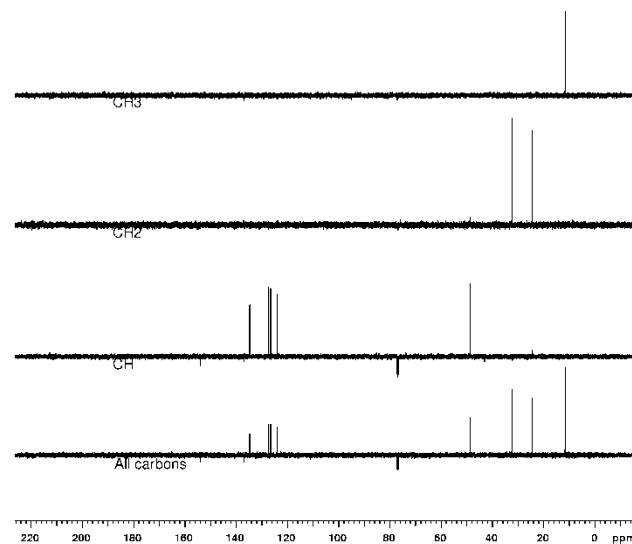
APT vs DEPT

In APT experiment quaternary carbon is also seen whereas in the DEPT only carbons attached to protons are seen. DEPT allows separation of carbons, thus aiding unambiguous identification.

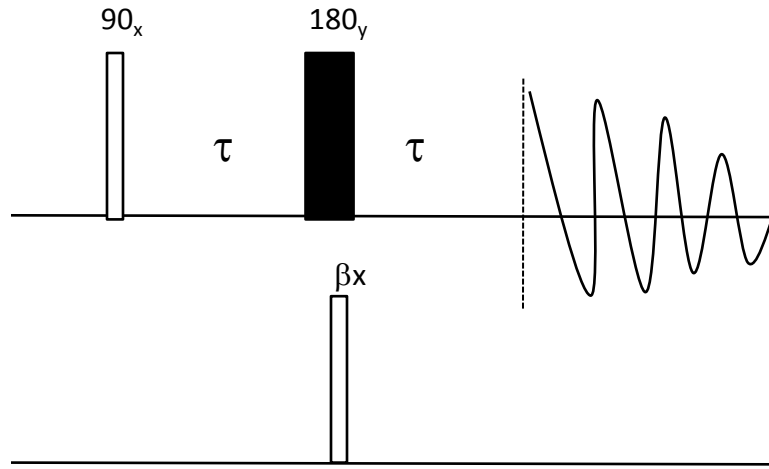
APT with $\theta=135^\circ$ CH and CH₃ are down; C and CH₂ are up.



DEPT

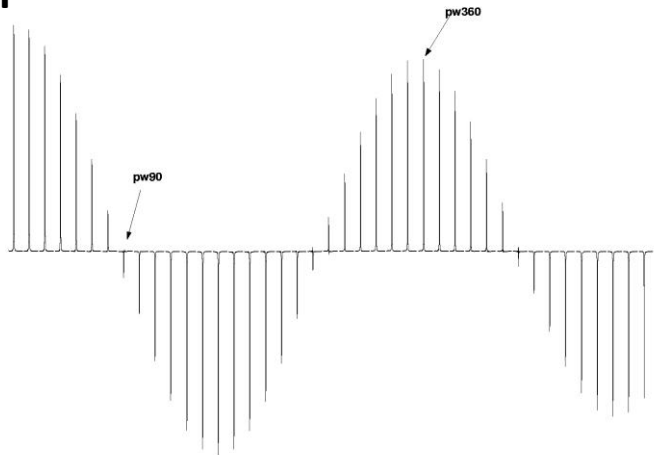


X-Nucleus PW Calibration



$\beta=0$ the signal is maximum.

$\beta=90^\circ$ there is no signal as the multiple quantum coherence is unobservable.



$$\tau = \frac{1}{2J}$$

$$-I_y$$



$$(\pi J_{IS}\tau)2I_zS_z$$

$$-I_y \cos(\pi J_{IS}\tau) + 2I_xS_z \sin(\pi J_{IS}\tau)$$

$$= 2I_xS_z$$



$$\beta S_x + \pi I_y$$

$$-2I_xS_z \cos \beta + 2I_xS_y \sin \beta$$



$$(\pi J_{IS}\tau)2I_zS_z$$

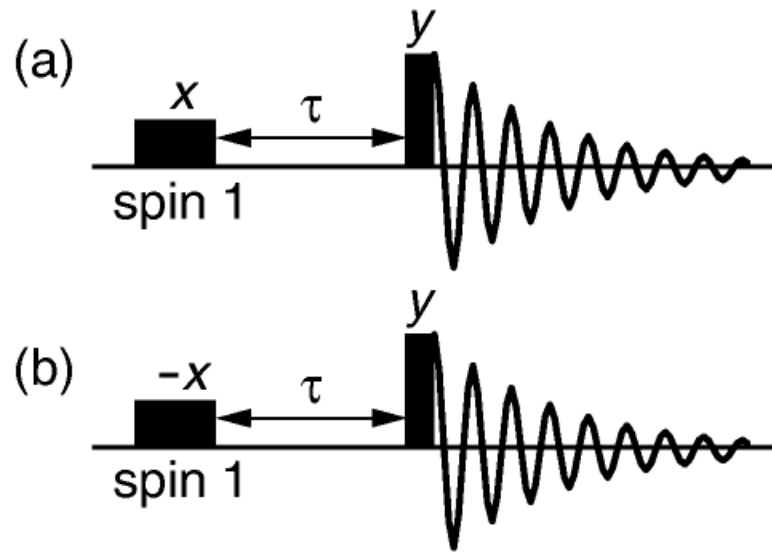
$$-I_y \cos \beta + 2I_xS_y \sin \beta$$

Coherence Transfer

Diagram illustrating Coherence Transfer in NMR. The top part shows a pulse sequence for ^1H and X channels. The ^1H channel has three pulses: a black pulse, a grey pulse, and another black pulse, with time intervals of $\frac{\Delta}{2}$ between them. A red circle labeled $\pm y$ is above the second pulse. The X channel has a single grey pulse followed by a black pulse, with a red circle labeled $\pm x$ above the black pulse. Below the pulses, an equation shows the transformation of magnetization. A blue arrow points from the initial state to the final state. The initial state is $-\gamma_I I_y$. The final state is $\gamma_I I_y \cos(\pi J_{IS} \Delta) - \gamma_I 2I_x S_z \sin(\pi J_{IS} \Delta)$. A red box highlights $2I_x S_z$. Below this, a blue arrow points to $\pm \frac{\pi}{2} I_y, \frac{\pi}{2} S_y$. The final state is then $\gamma_I I_y \cos(\pi J_{IS} \Delta) \pm \gamma_I 2I_z S_x \sin(\pi J_{IS} \Delta)$. A red box highlights $2I_z S_x$.

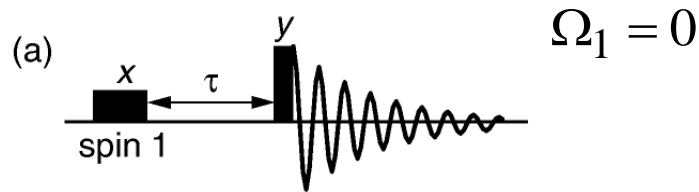
The conversion of $2I_x S_z$ to $2I_z S_x$ is an important aspect of pulsed NMR and is known as the **coherence transfer** step. Coherence transfer is extensively used to obtain coupling network information in multi dimensional NMR.

Coherence Transfer – Selective Correlation Experiment



Let us consider two protons coupled to each other and in the experiment (a) we apply a selective 90° x -pulse on spin 1 and after a delay τ apply a non selective 90° pulse along y that rotates both protons. In (b) we do the same but the selective pulse on spin 1 is applied along $-x$. Also say that we are on resonance to spin 1 i.e. $\Omega_1=0$.

Coherence Transfer – Selective Correlation Experiment



$$I_{1z} + I_{2z}$$

$$\downarrow \frac{\pi}{2} I_{1x}$$

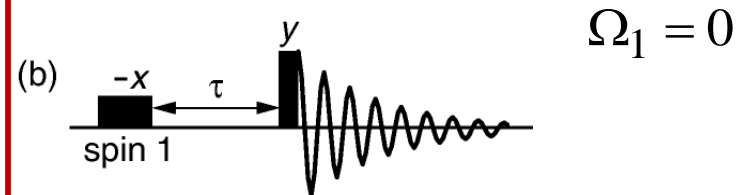
$$-I_{1y} + I_{2z}$$

$$\downarrow \pi J_{12} \tau 2 I_{1z} I_{2z}$$

$$-I_{1y} \cos(\pi J_{12} \tau) + 2 I_{1x} I_{2z} \sin(\pi J_{12} \tau) + I_{2z}$$

$$\downarrow \frac{\pi}{2} (I_{1y} + I_{2y})$$

$$-I_{1y} \cos(\pi J_{12} \tau) - 2 I_{1z} I_{2x} \sin(\pi J_{12} \tau) - I_{2x}$$



$$I_{1z} + I_{2z}$$

$$\downarrow -\frac{\pi}{2} I_{1x}$$

$$+I_{1y} + I_{2z}$$

$$\downarrow \pi J_{12} \tau 2 I_{1z} I_{2z}$$

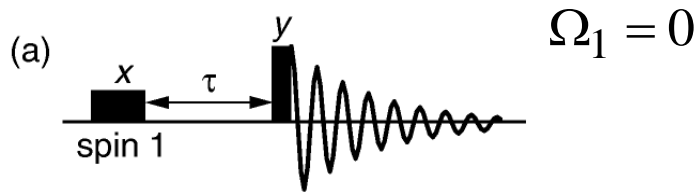
$$+I_{1y} \cos(\pi J_{12} \tau) - 2 I_{1x} I_{2z} \sin(\pi J_{12} \tau) + I_{2z}$$

$$\downarrow \frac{\pi}{2} (I_{1y} + I_{2y})$$

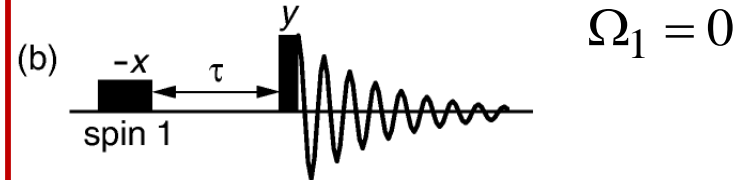
$$+I_{1y} \cos(\pi J_{12} \tau) + 2 I_{1z} I_{2x} \sin(\pi J_{12} \tau) - I_{2x}$$

$$(b) - (a) = I_{1y} \cos(\pi J_{12} \tau) + 2 I_{1z} I_{2x} \sin(\pi J_{12} \tau)$$

Coherence Transfer – Selective Correlation Experiment



$$-I_{1y} \cos(\pi J_{12}\tau) - 2I_{1z}I_{2x} \sin(\pi J_{12}\tau) - I_{2x}$$



$$+I_{1y} \cos(\pi J_{12}\tau) + 2I_{1z}I_{2x} \sin(\pi J_{12}\tau) - I_{2x}$$

$$(c) = (b) - (a) = I_{1y} \cos(\pi J_{12}\tau) + 2I_{1yz}I_{2x} \sin(\pi J_{12}\tau)$$

An uncoupled third spin

