NMR Spectroscopy: Principles and Applications

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2D NMR – Heteronuclear 2D

Lecture 7

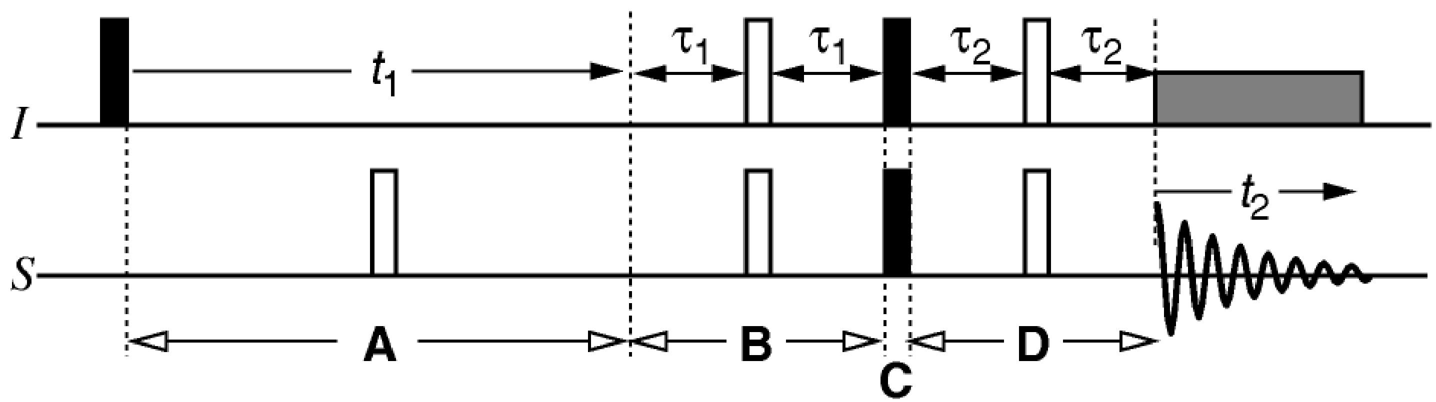
Hetero Nuclear 2D-NMR

Two dimensional NMR can be used to correlate NMR signals arising from different nuclei such as ^{13}C, ^{15}N, ^{31}P etc with the attached protons. We have already seen 1D-editing experiments like APT, INEPT, and DEPT that transfer coherences from ^{1}H to ^{13}C.

Since the correlated nuclei are of different type usually we will represent one species as spin I and the other as spin S.

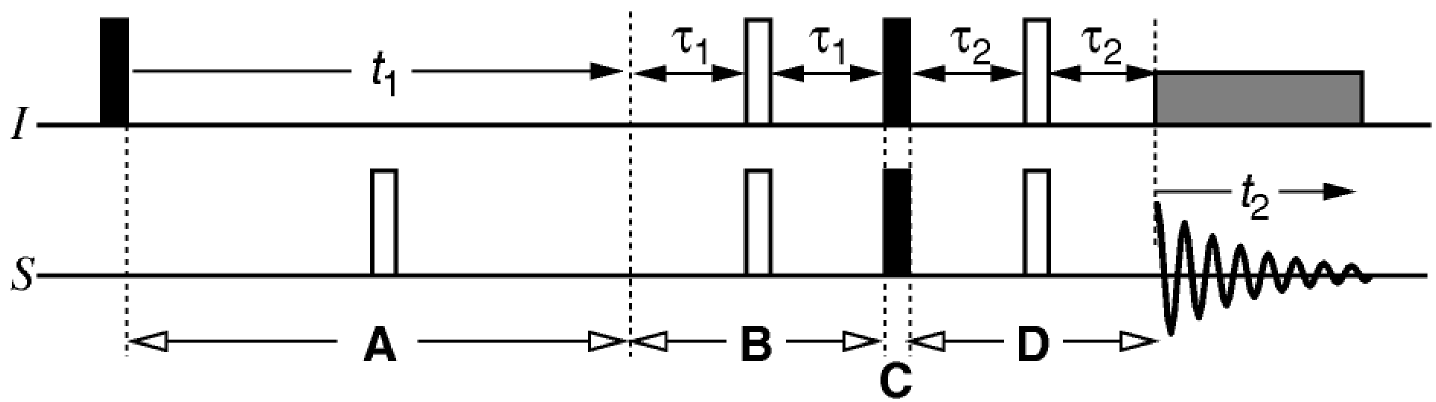
HETCOR - experiment

A COSY experiment when correlating two different type of nuclei is called Heteronuclear Correlation (HETCOR) experiment. The pulse sequence is shown below. Usually, spin I is ^{1}H and the shifts of which in ω_1 is correlated with spin S, the attached hetero nuclei, that is detected in the t_2 period whose chemical shift appear in ω_2 dimension.



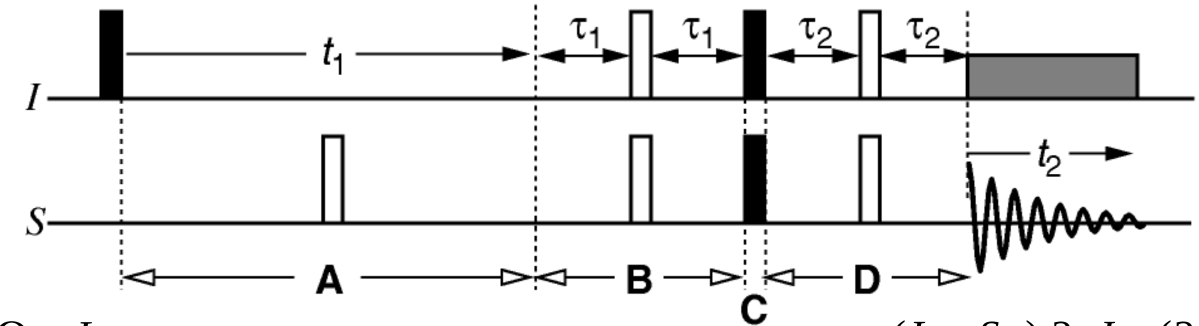
HETCOR - experiment

Spin I coherences are excited by the first I spin 90^o pulse that labels its offset in t_1 (period A). The subsequent spin echo sequence (period B) converts the I-spin in-phase coherence into I-spin anti-phase coherence with respect to spin S. The subsequent two 90^o pulses effect the coherence transfer to anti-phase S-spin coherence (period C) which is converted into S-spin in-phase coherence by the spin echo sequence (period D) and detected during t_2.



HETCOR - experiment

It is instructive to go through the spin evolution to contrast HETCOR from COSY .



$$I_z \xrightarrow{\frac{\pi}{2}I_x} -I_{1y} \xrightarrow{\Omega_I t_1 I_{1z}} -I_y \cos(\Omega_I t_1) + I_x \sin(\Omega_I t_1) \xrightarrow{\pi(I_x+S_x),2\pi J_{IS}(2\tau_1)I_z S_z}$$

$$\cos(\Omega_I t_1)\cos(\pi J_{IS}\,2\tau_1)I_y - \cos(\Omega_1 t_1)\sin(\pi J_{IS}\,2\tau_1)2I_x S_z$$

$$+\sin(\Omega_1 t_1)\cos(\pi J_{IS}\,2\tau_1)I_x + \sin(\Omega_1 t_1)\sin(\pi J_{IS}\,2\tau_1)2I_y S_z$$

$$\xrightarrow{\frac{\pi}{2}I_x+\frac{\pi}{2}S_x}$$

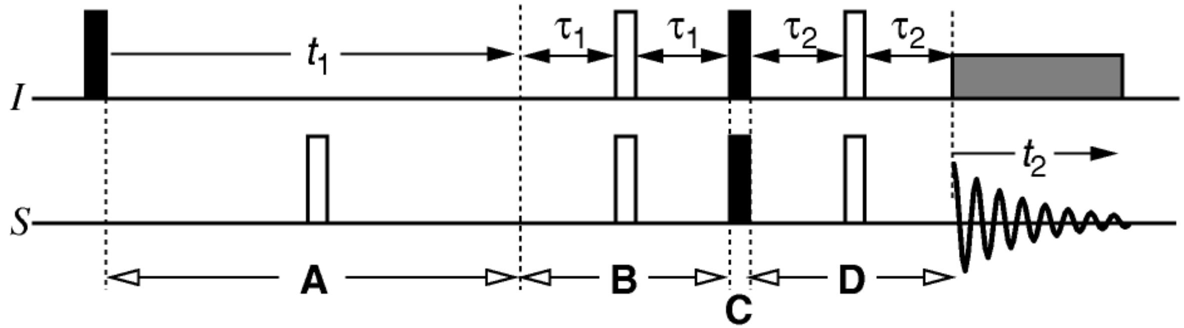
$$\cos(\Omega_I t_1)\cos(\pi J_{IS}\,2\tau_1)I_{1z} + \cos(\Omega_I t_1)\sin(\pi J_{IS}\,2\tau_1)2I_x S_y$$

$$+\sin(\Omega_I t_1)\cos(\pi J_{IS}\,2\tau_1)I_x \boxed{-\sin(\Omega_I t_1)\sin(\pi J_{IS}\,2\tau_1)2I_z S_y}$$

At the end of period C

HETCOR - experiment

Since we detect only spin S, the S-spin coherence is the only relevant term during period D and t_2 period.



$$-\sin(\Omega_I t_1)\sin(\pi J_{IS} 2\tau_1) 2I_z S_y \xrightarrow{\pi(I_x+S_x),2\pi J_{IS}(2\tau_2)I_z S_z}$$

$$\boxed{\sin(\Omega_I t_1)\sin(\pi J_{IS} 2\tau_1)\sin(\pi J_{IS} 2\tau_2) S_x}$$

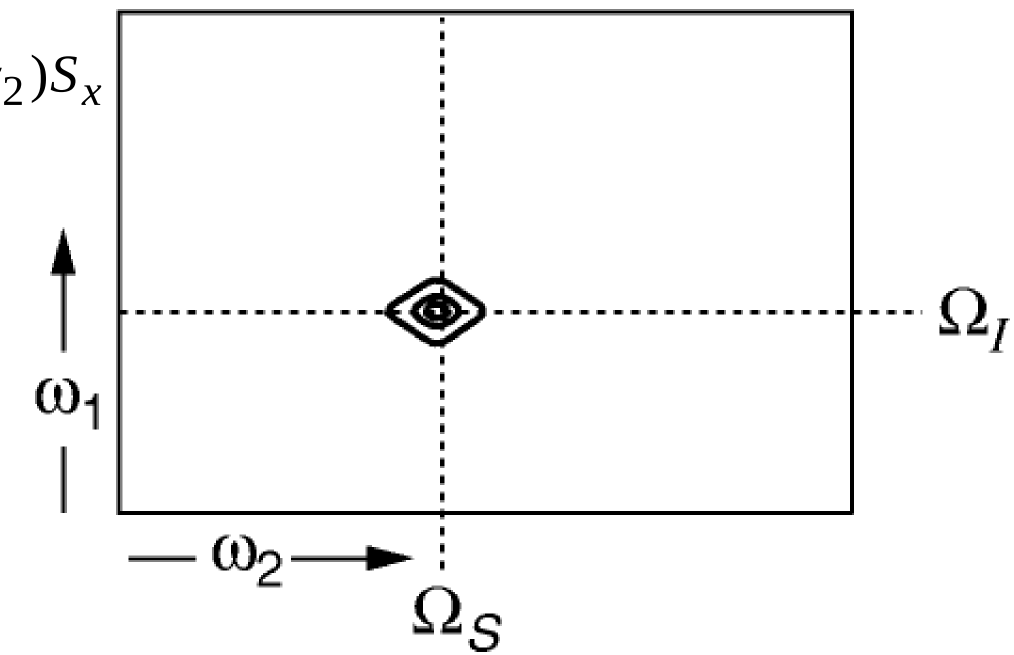
At the end of period D or at the start of t_2.

In t_2 the S-spin coherence evolve with its own offset. The coupling to I spin is removed by the decoupling field. Also note that in t_1 also there is no evolution of the coupling term – only the offset of spin-I is labeled.

HETCOR - experiment

The schematic 2D spectrum can be represented as below.

$$\sin(\Omega_I t_1)\sin(\pi J_{IS}\, 2\tau_1)\sin(\pi J_{IS}\, 2\tau_2)S_x$$



A peak at Ω_I in ω_1 and Ω_S in ω_2 appear. It is the cross peak equivalent of the COSY spectrum. There is no diagonal peak as we are correlating two different nuclei.

HSQC - experiment

In HETCOR experiment, the magnetization starts from spin I and transferred to spin S and the S spins are detected. Thus, there is a signal enhancement of γ_I/γ_S as we have seen in the 1D lecture of INEPT and DEPT schemes.

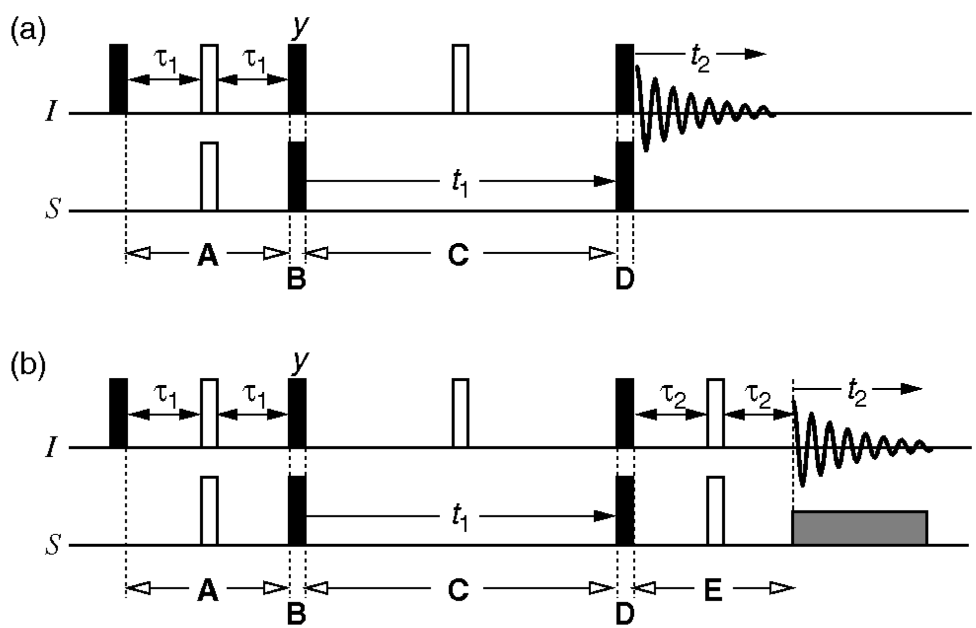
$$\sin(\Omega_I t_1)\sin(\pi J_{IS} 2\tau_1)\sin(\pi J_{IS} 2\tau_2)S_x$$

Instead of detecting the low γ nucleus, we can start as in HETCOR and frequency label S-spin coherences in t_1 and detect I-spin by an extra INEPT back transfer , we get a new experiment called Heteronuclear Single quantum Correlated Spectroscopy (HSQC). We not only get the γ_I/γ_S advantage, but also additional higher sensitivity in the ratio $(\gamma_I/\gamma_s)^{3/2}$ by detecting the I spins.

$$\frac{S}{N} \propto \gamma_I \gamma_I^{3/2}$$

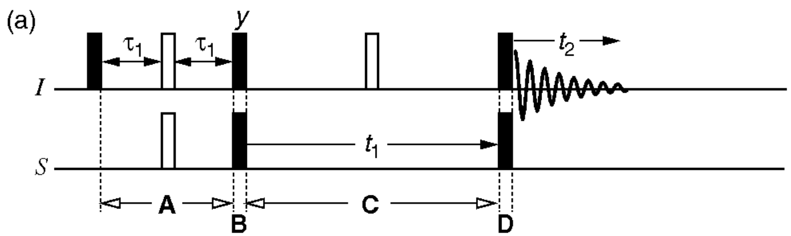
HSQC - experiment

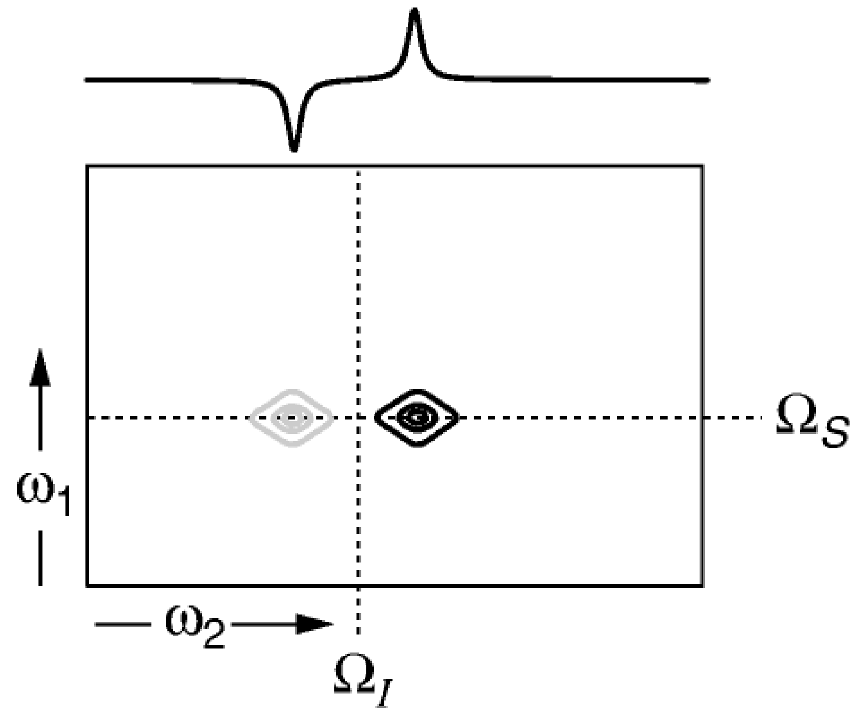
The HSQC pulse sequence is given below: (a) The INEPT sequence (part A +B) creates S-spin antiphase coherence with respect to spin I and the S-spin coherence is frequency labeled during t_1 (period C). The final two 90^o pulses converts the S-spin antiphase coherence to I-spin antiphase coherence and the detection of I spins start immediately. In (b) after period D another spin echo is added to refocus the I spin coherence so that decoupling can be used. Period D+E is known as reverse INEPT.



HSQC - Experiment

We start at the end of period A





$$\cos(\pi J_{IS}2\tau_1)I_y - \sin(\pi J_{IS}2\tau_1)2I_xS_z$$

$$\xrightarrow{\frac{\pi}{2}I_y+\frac{\pi}{2}S_x}$$

$$\cos(\pi J_{IS}2\tau_1)I_{1y} - \sin(\pi J_{IS}2\tau_1)2I_zS_y$$

$$\xrightarrow{\pi I_x,\Omega_S t_1 S_z}$$

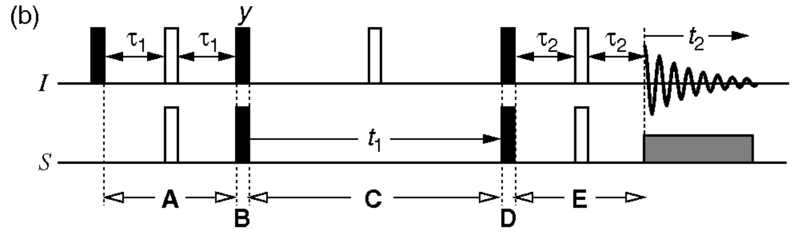
$$-\cos(\pi J_{IS}2\tau_1)I_{1y} - \sin(\pi J_{IS}2\tau_1)\{-2I_zS_y\cos(\Omega_S t_1) + 2I_zS_x\sin(\Omega_S t_1)\}$$

$$\xrightarrow{\frac{\pi}{2}I_x+\frac{\pi}{2}S_x}$$

$$-\cos(\pi J_{IS}2\tau_1)I_{1z} \boxed{-\sin(\pi J_{IS}2\tau_1)(2I_yS_z\cos(\Omega_S t_1)} + \sin(\pi J_{IS}2\tau_1)2I_yS_x\sin(\Omega_S t_1))$$

HSQC - Experiment

We start at the end of period D

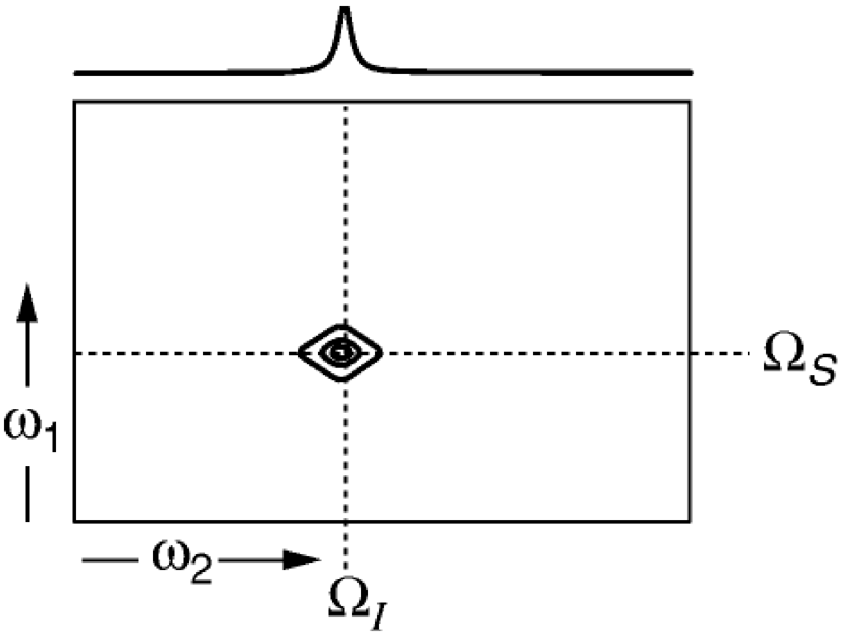


$$-\sin(\pi J_{IS}\, 2\tau_1) 2I_y S_z \cos(\Omega_S t_1)$$

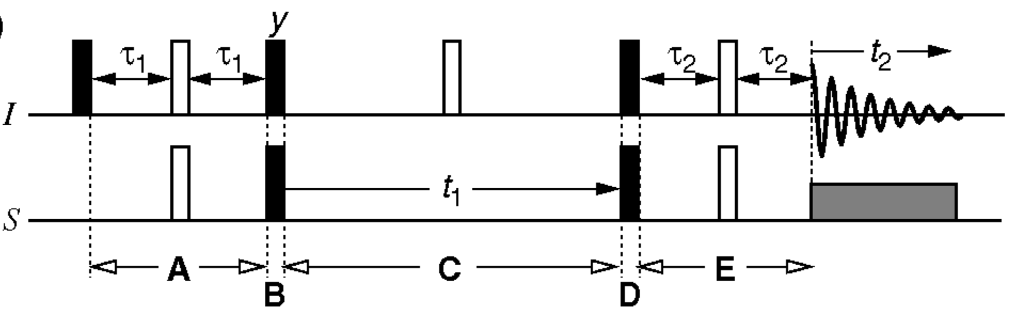
$$\xrightarrow{\textit{Period E}}$$

$$-\sin(\pi J_{IS}\, 2\tau_1)\cos(\pi J_{IS}\, 2\tau_2)\cos(\Omega_S t_1) 2I_y S_z$$

$$+\sin(\pi J_{IS}\, 2\tau_1)\sin(\pi J_{IS}\, 2\tau_2)\cos(\Omega_S t_1) I_x$$



2D-HSQC-Summary



$$\tau_1 = \tau_2 = \frac{1}{4J_{IS}}$$

$$-I_y$$

$$\downarrow \pi I_x + \pi S_x \quad (\pi J_{IS} 2\tau_1) 2I_z S_z$$

$$I_y \cos(\pi J_{IS} 2\tau_1) - 2I_x S_z \sin(\pi J_{IS} 2\tau_1)$$

$$= -2I_x S_z$$

$$\downarrow \frac{\pi}{2} I_y + \frac{\pi}{2} S_x$$

$$-2I_z S_y$$

$$\downarrow \pi I_x, (\omega_s S_z) t_1$$

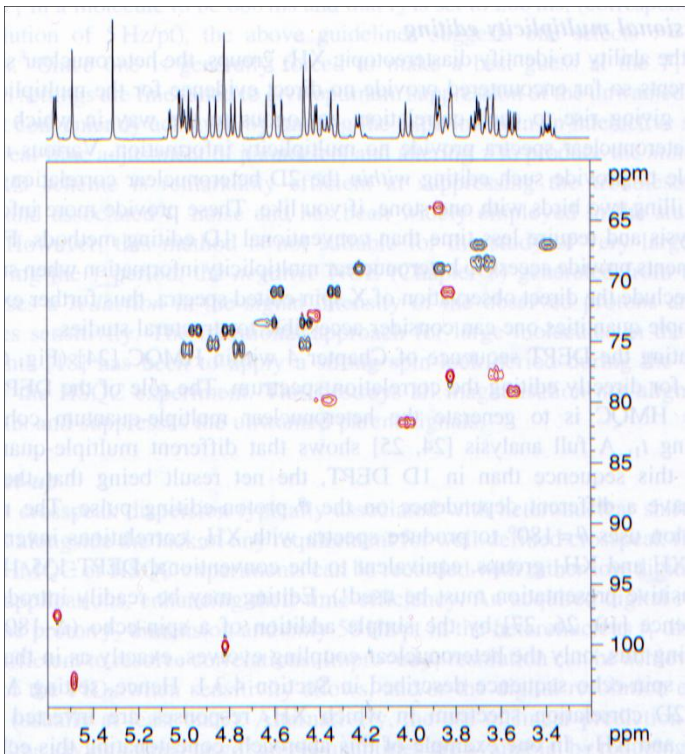
$$-2I_z S_y \cos(\omega_s t_1) + 2I_z S_x \sin(\omega_s t_1)$$

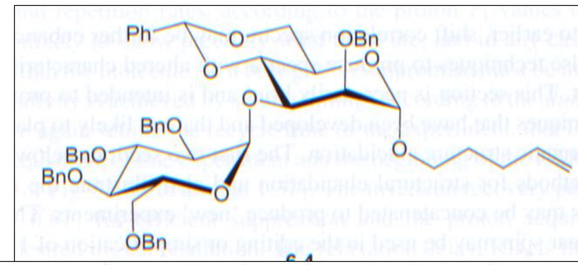
$$\downarrow \frac{\pi}{2} I_x + \frac{\pi}{2} S_x$$

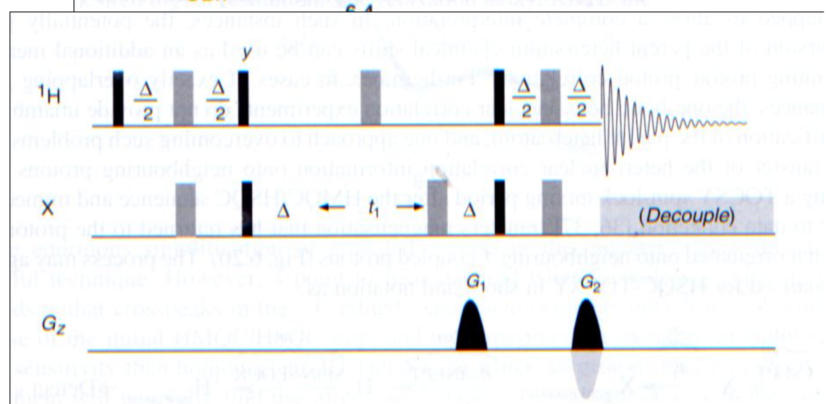
$$I_x \xleftarrow[\pi I_x + \pi S_x]{(\pi J_{IS} 2\tau_2) 2I_z S_z} -2I_y S_z \cos(\omega_s t_1) - 2I_y S_x Sin(\omega_s t_1)$$

Examples - HSQC

^{1}H-^{13}C (natural abundance) multiplicity edited HSQC of disaccharide is shown below.



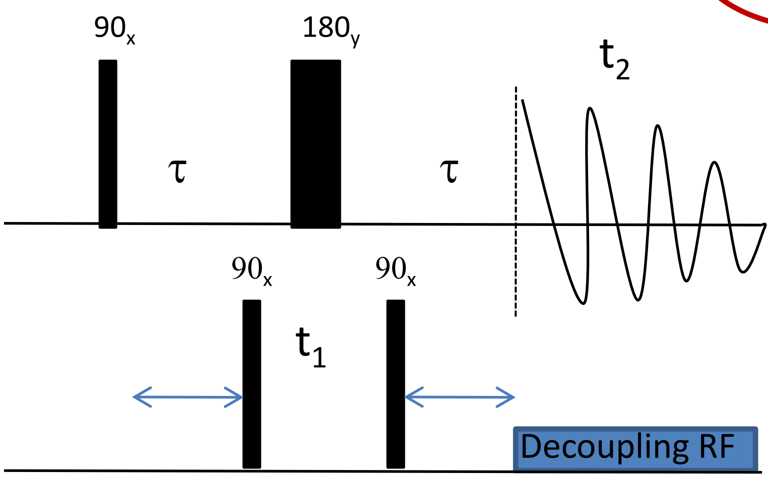




Δ=1/2J inverts XH_2 (black) responses relative to XH and XH_3 (red)

2D-Heteronuclear Multiple Quantum Coherence Spectroscopy (HMQC)

$$\tau = \frac{1}{2J_{IS}}$$



$$-I_y$$

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

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

$$= 2I_xS_z$$

$$\downarrow \frac{\pi}{2}S_x$$

Multiple Quantum Coherence → $2I_xS_y$

$$\downarrow \pi I_y, (\omega_s S_z)t_1$$

$$2I_xS_y\cos(\omega_s t_1) - 2I_xS_x\sin(\omega_s t_1)$$

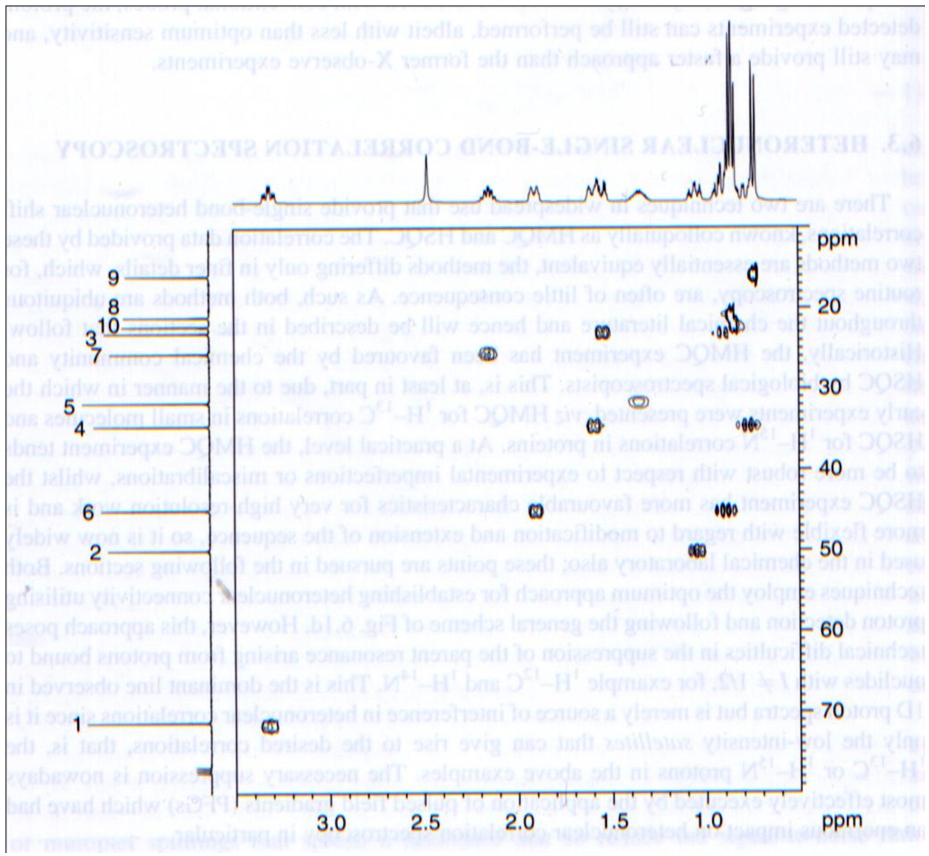
$$\downarrow \frac{\pi}{2}S_x$$

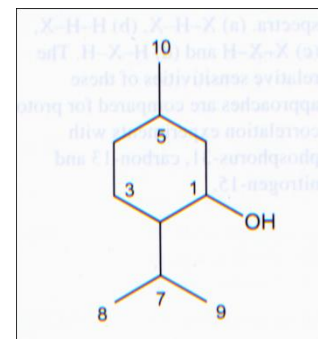
$$2I_xS_z\cos(\omega_s t_1) + 2I_xS_x Sin(\omega_s t_1)$$

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

Examples - HMQC

^{1}H-^{13}C (natural abundance) HMQC of Menthol is shown below.



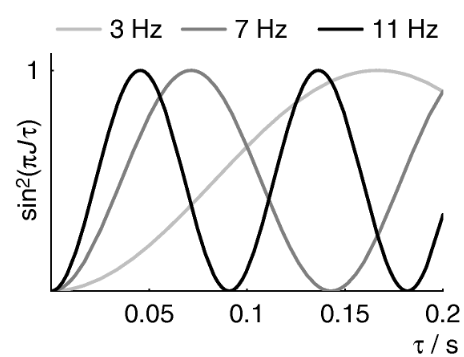


Long Range Correaltion- Hetero Nuclear Multibond Correlation (HMBC)

In both HSQC and HMQC the τ *delays are set based on one-bond J coupling constant values. If we want to observe long range couplings across multiple bonds then their values are much smaller and vary over a wide range. In HMQC, there are two* τ *delays and the peak intensity thus depend as*

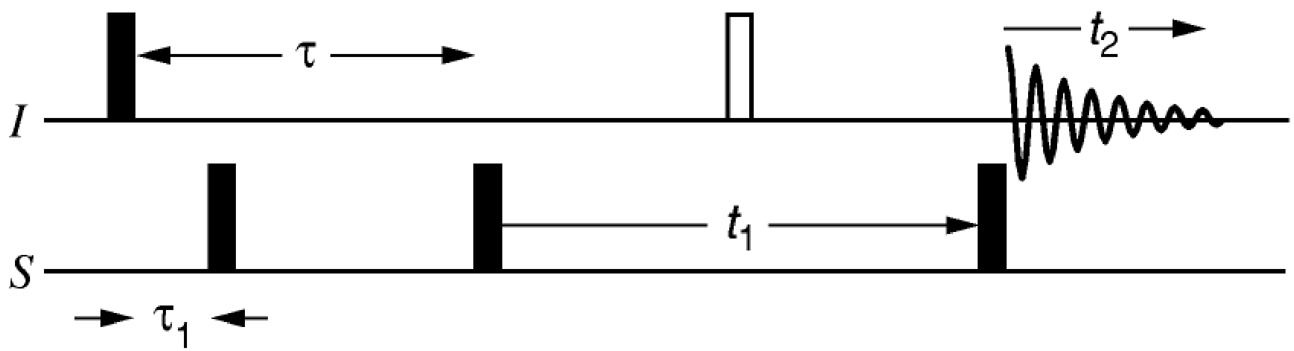
$$\sin^2(\pi J \tau)$$

By plotting this function for various coupling constants we can optimize the τ *delay to observe long range coupling and hence the remote cross peaks in a 2D spectrum. Such an experiment is known as HMBC.*



Long Range Correaltion- Hetero Nuclear Multibond Correlation (HMBC)

In HMBC experiment, we look for only the long range coupling peaks and suppress the direct peaks that we see in a HMQC or HSQC spectrum by using the sequence below.

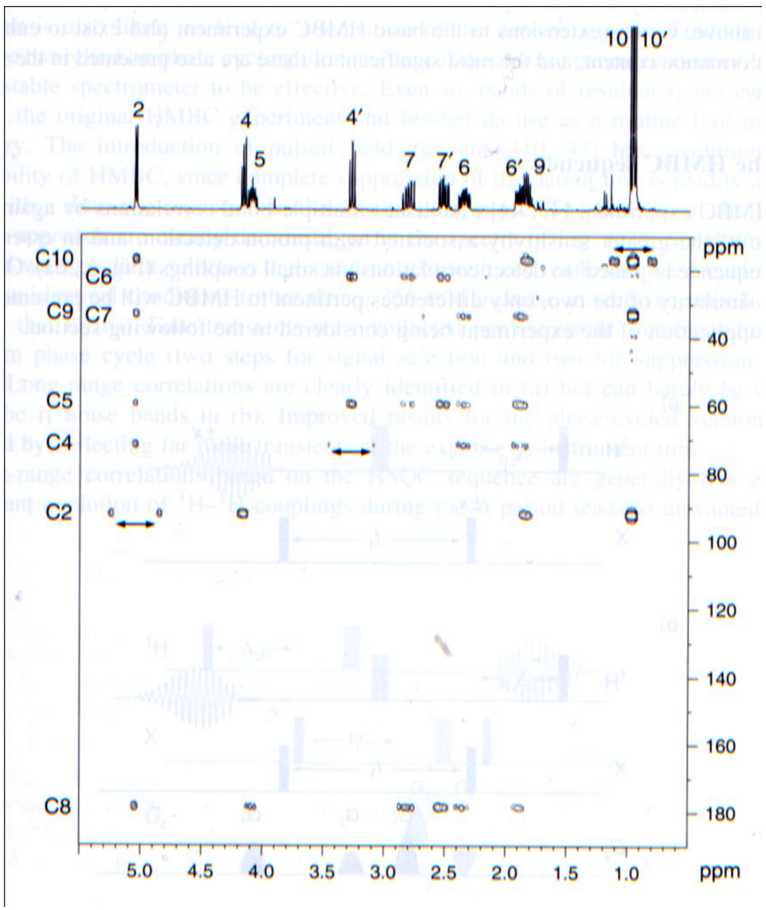


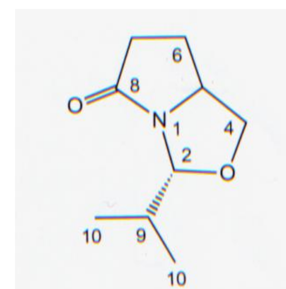
The delay τ_1 is set to $1/2(^1J_{IS})$ and t is the usual long delay corresponding to long range coupling. The one bond coupling generate its own antiphase coherence that is converted into MQC by the first 90o pulse on the S-spin. The experiment is done twice once with this pulse along (x)-axis and again along (-x)-axis and added to suppress the direct peak.

$$\tau_1 = \frac{1}{2\,^1J_{IS}} = \frac{1}{2*160} = 3.125ms \quad \tau = 20*3.125ms = 62.5ms$$

Examples - HMBC

^{1}H-^{13}C (natural abundance) HMBC is shown below.





HMQC and HMBC

A typical HMQC (Left) and HMBC (Right) of ^{1}H-^{13}C correlation spectra will look as below.