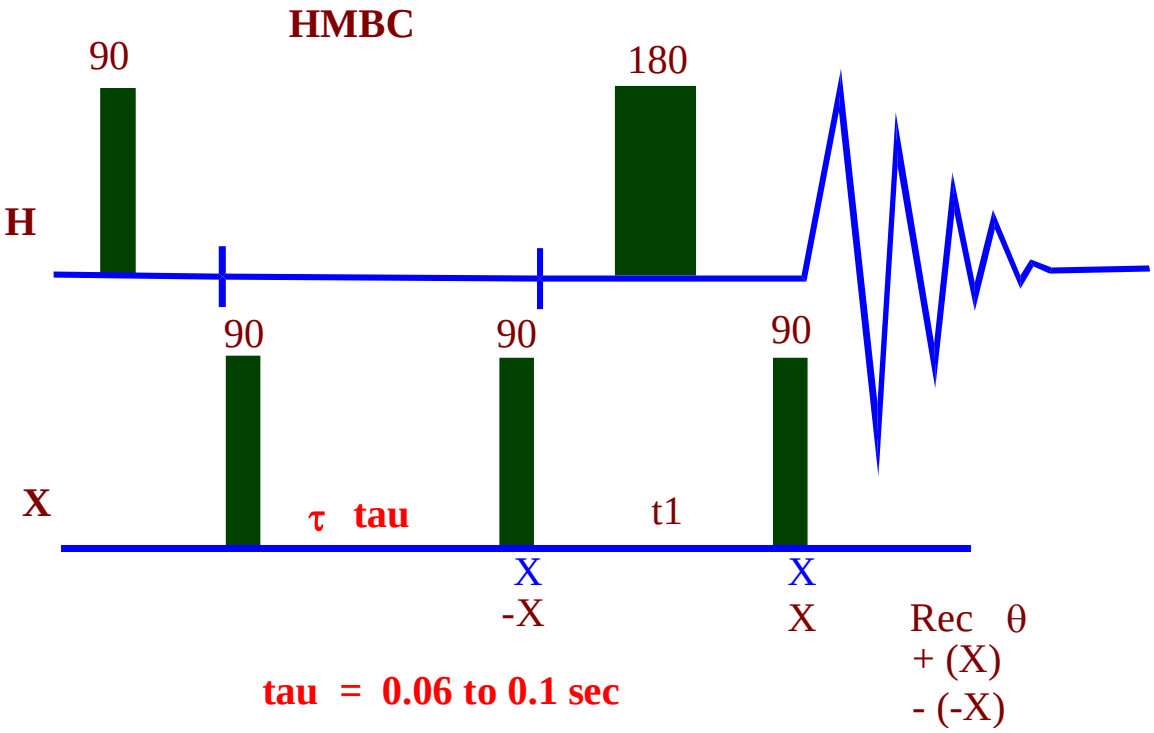
gHMBC experiment



Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple Bond Coherence (HMBC) are 2-dimensional inverse H,C correlation techniques that allow for the determination of carbon (or other heteroatom such as 15N) to hydrogen connectivity.

HMQC is selective for direct C-H coupling and HMBC will give longer-range couplings (2-4 bond coupling). HMBC (gHMBC) improves the acquired spectrum by significantly reducing unwanted signal artifacts.

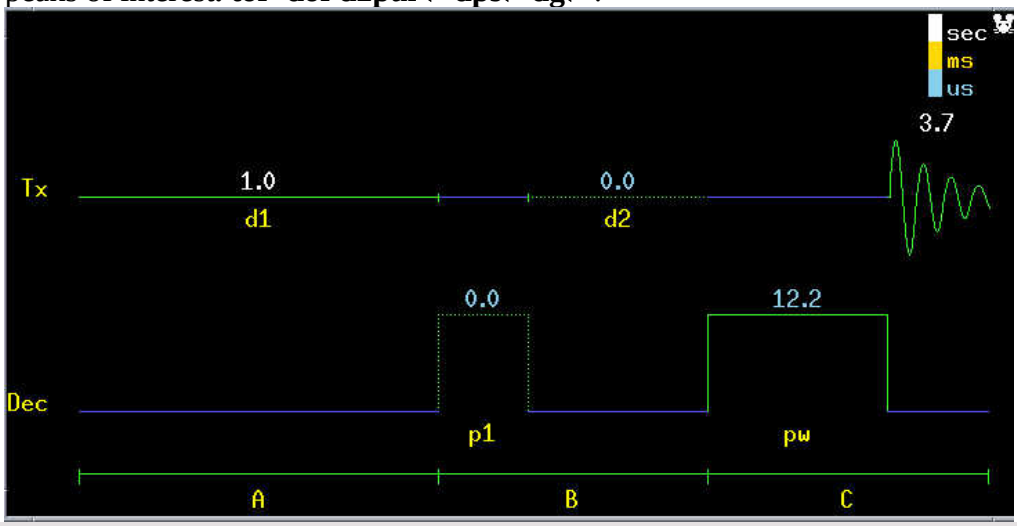
The HMBC experiment detects long range coupling between proton and carbon (two or three bonds away) with great sensitivity. The length of the tau delay can be adjusted to detect relatively large coupling constants (4-10 Hz) tau = 0.06 s or smaller couplings (2-7 Hz) tau = 0.1 s.
In this sequence, the first 90°-pulse on Carbon-13 serves as a low-pass filter that suppresses one-bond correlation and passes the smaller coupling. This pulse creates multiple quantum coherence for the one-bond coupling, which is removed from the spectra by alternating the phase of the Carbon-13 pulse. The second 90°-pulse on C-13 creates multiple quantum coherence for the long-range couplings. After the evolution time t1, the magnetization is converted back into detectable single quantum proton

magnetization. The carbon decoupler is never used in this sequence: therefore the protons displays homonuclear as well as heteronuclear couplings.

This technique is very valuable to detect indirectly quaternary carbons coupled to protons - specially useful if direct Carbon-13 is impossible to obtain due to low amount of material available. This very useful sequence provides information about the skeleton of a molecule. It could be an alternative to the 2D-INADEQUATE experiment (which is so insensitive). It is also very useful in carbohydrate area as a sequence analysis tool that provides unique information concerning connectivities across glycosidic linkages. Another area of interest for using HMBC is in the peptide-protein area - specially when applied to a 15N labeled protein - It is possible with this technique to get connectivities between the Nitrogen and the CHα proton of the amino acid of the next residue.

Steps of doing gHMBC

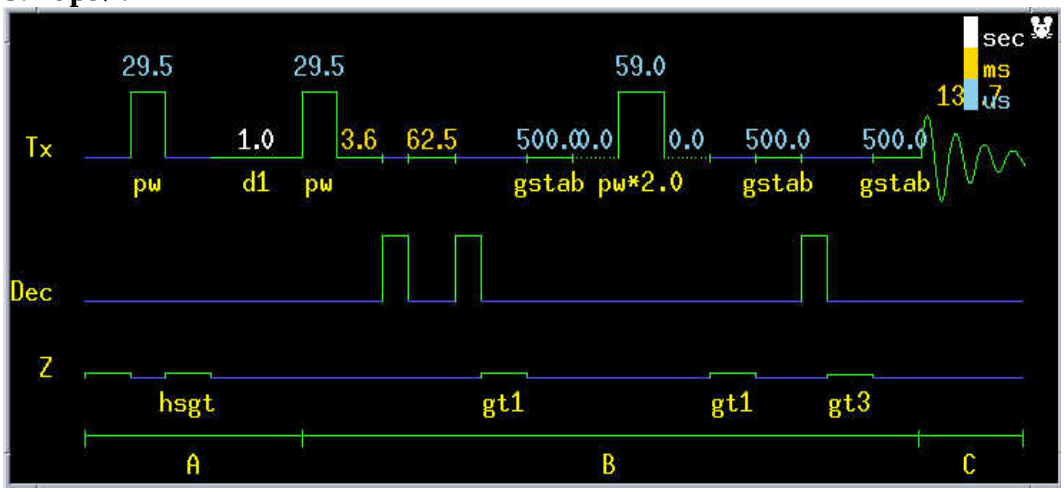
1. Acquire a 1D proton spectrum. Narrow the 1H spectral width to include all the peaks of interest**. tof=dof d2pul ↵ dps↵ dg↵** .



ACQUISITION		SAMPLE		PROCESSING		FLAGS	
sfrq	399.730	date	Jul 1 2004	lb	not used	il	n
tn	H1	solvent	D2O	sb	not used	in	n
at	3.744	file	exp	gf	not used	dp	y
np	44932	DECOUPLING		awc	not used	hs	nn
sw	6000.6	dn	H1	lsfid	not used	SPECIAL	
fb	3000	dof	-154.4	phfid	not used	temp	not used
bs	16	dm	nnn	wtfile			
ss	0	dmm	c	proc	ft		
tpwr	63	dmf	11148	fn	not used		
pw	12.2	dpwr	42	math	f		
p1	0	homo	n				
d1	1.000			werr			
d2	0			wexp			
tof	-154.4			wbs			
nt	1			wnt			
ct	0						

2. **dn=13C tpwr=57↵**.

3. Array ***pw90***. **pw=pw90** ↵. Remember the **tpwr** should be the same as in 2D.
4. **mf(1,2) jexp2 gHMBC**↵. Turn off the spinner.
5. **dps**↵.

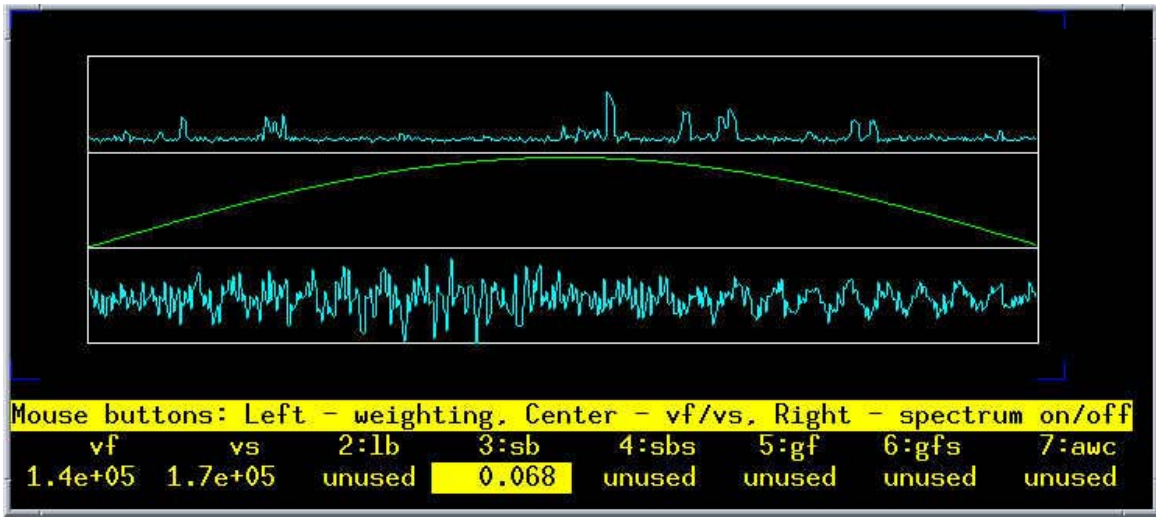


6. **dg**↵.

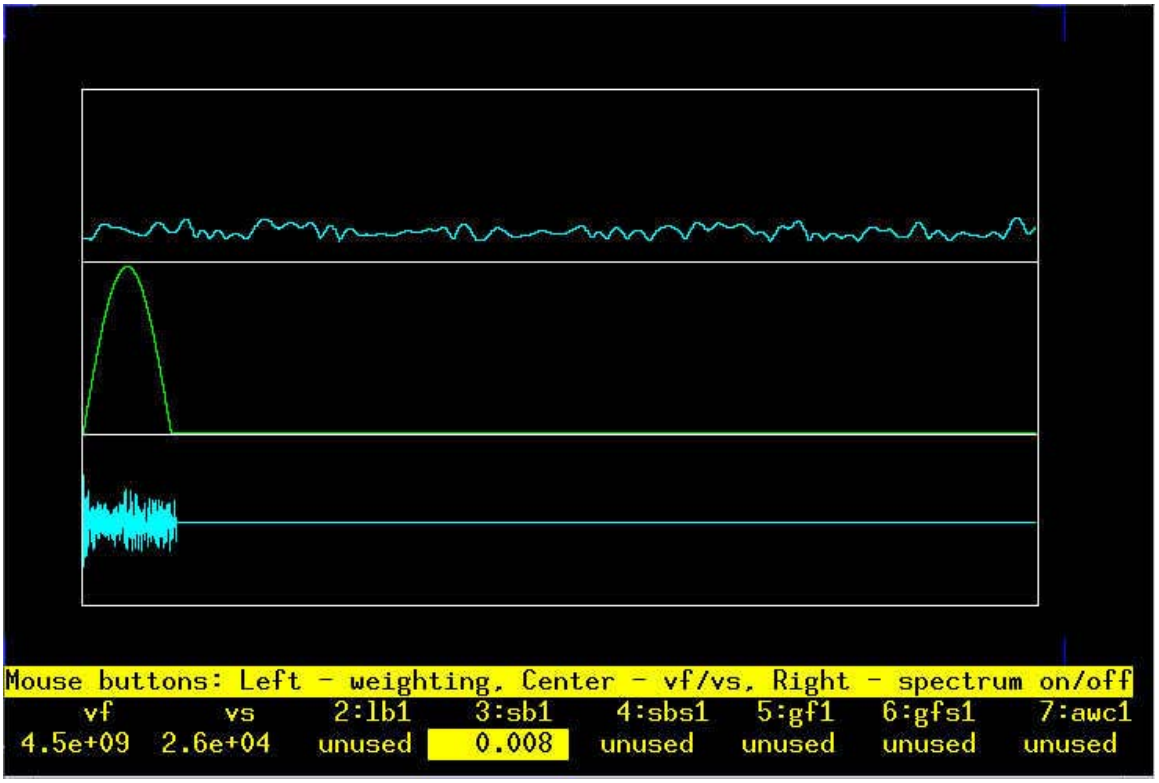
ACQUISITION		TRANSMITTER		HMBC		PROCESSING	
sw	3773.6	tn	H1	j1xh	140.0	sb	0.068
at	0.136	sfrq	399.729	jnxh	8.0	sbs	not used
np	1024	tof	-308.2	GRADIENTS		fn	1024
bs	8	tpwr	57	gzlvl1	4854	2D PROCESSING	
ss	8	pw	29.500	gt1	0.001000	sb1	0.008
d1	1.000	DECOUPLER		gzlvl3	2427	sbs1	not used
nt	16	dn	C13	gt3	0.001000	fn1	2048
ct	16	dof	1042.6	gstab	0.000500	SAMPLE	
2D ACQUISITION		pwxlvl	57	hsglvl	4854	date	Apr 7 2004
sw1	24125.5	pwx	9.300	hsgt	0.002000	solvent	CDCl3
ni	400	dm	nnn	SPECIAL		sample	undefined
		dmm	ccc	temp	not used		
		dmf	18519	spin	not used		
		dpwr	41	gain	40		
				pw90	29.500		
				sspul	y		

7. Set the **sw1**, carbon sweep width. This is supposed to be from a normal 13C 1D NMR. The sw1 should encompass all the carbons. To do this, run C-13 NMR in exp3 and place cursors on both sides and type **movesw**. The new **sw** is the **sw1** in ***HMBC***. Go back to exp2.
8. Set **dof** which is **tof** of 1D C-13 NMR.
9. Array ***pwx***. To do this, type **ni=1 nt=4 ai wexp='wft dssh'** ↵ then do the same work as you did on **pw90** for a 1D spectrum. You should see the **pwx360. pwx=pwx360/4**↵.
10. Set ***gain***. Type **gain?**↵ **gain=XXX**↵ **(**the value found by the computer in the previous spectrum).
11. **nt=16 ni=400**↵ (***ni~sw1/60, dres1=sw1/2ni ~ 30 Hz/pt*** is sufficient).
12. **go**↵
13. **svf('gHMBC_molecule')** ↵.
14. **sb=-at/2 sbs=sb sb1=-ni/2sw1 sbs1=sb1**.

15. Or **wft(1) wti**↵, then adjust the sine bell. But normally ***sb=-at/2*** is already very good.

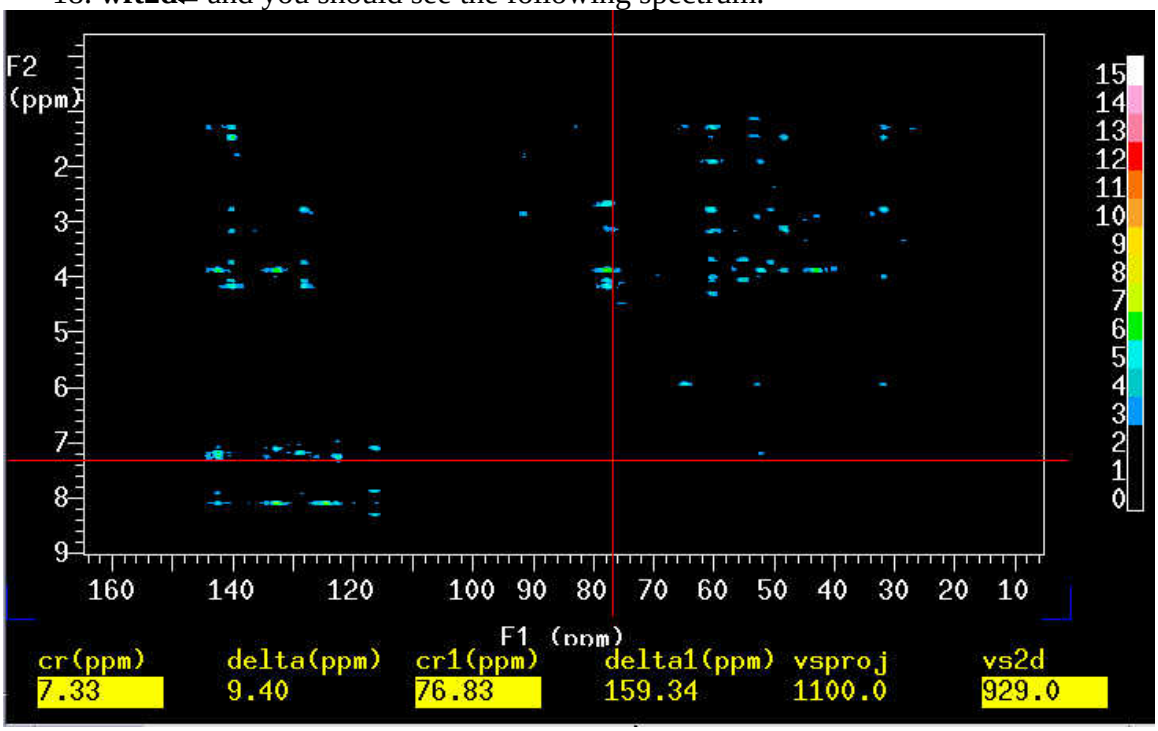


16. **wft1d**↵ click on ***VS+20%*** if you can't see anything. You see the ferogyrogram vertically (F2,t1). **trace='f1'** ↵ **dconi**↵ you see the ferogyrogram horizonally.
17. Click on ***trace*** and then **wti**↵.



Adjust the ***sb1*** to the end of the FID. Normally it should be close to ***–ni/1sw1***.

18. **wft2d**↵ and you should see the following spectrum.



19. **pcon(10,1.2) page**.
20. to set up linear prediction, type **setLP1**, this will change ***fn1*** to a new value say 8192, **wft2d**↵**.**