LECTURE NOTES CHEM 781

PART 6: Two dimensional NMR -basic theory and through bond connectivity

November 11, 2008

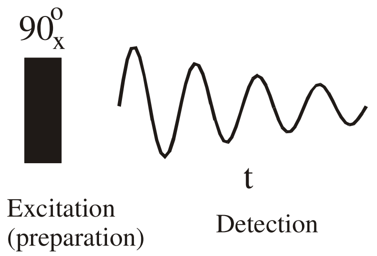
1 The prototype 2D experiment

so far we transferred proton magnetization to ^{13}C to enhance sensitivity. We also were able to retain limited coupling information in decoupled ^{13}C spectrum.

New goal: also obtain chemical shift of coupling partner

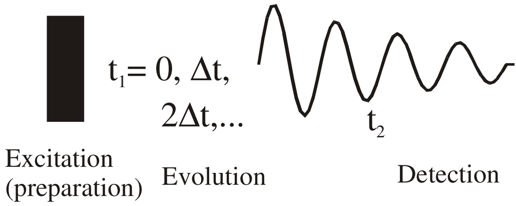
=> The detected signal has to be made dependent on chemical shift taking place prior to detection

Simplest example is adding delay t_1 before acquisition and incrementing this delay in series of one pulse experiments:



$-M_0\, I_y \cos(\Omega t)$

one time dependent factor gives one frequency after FT



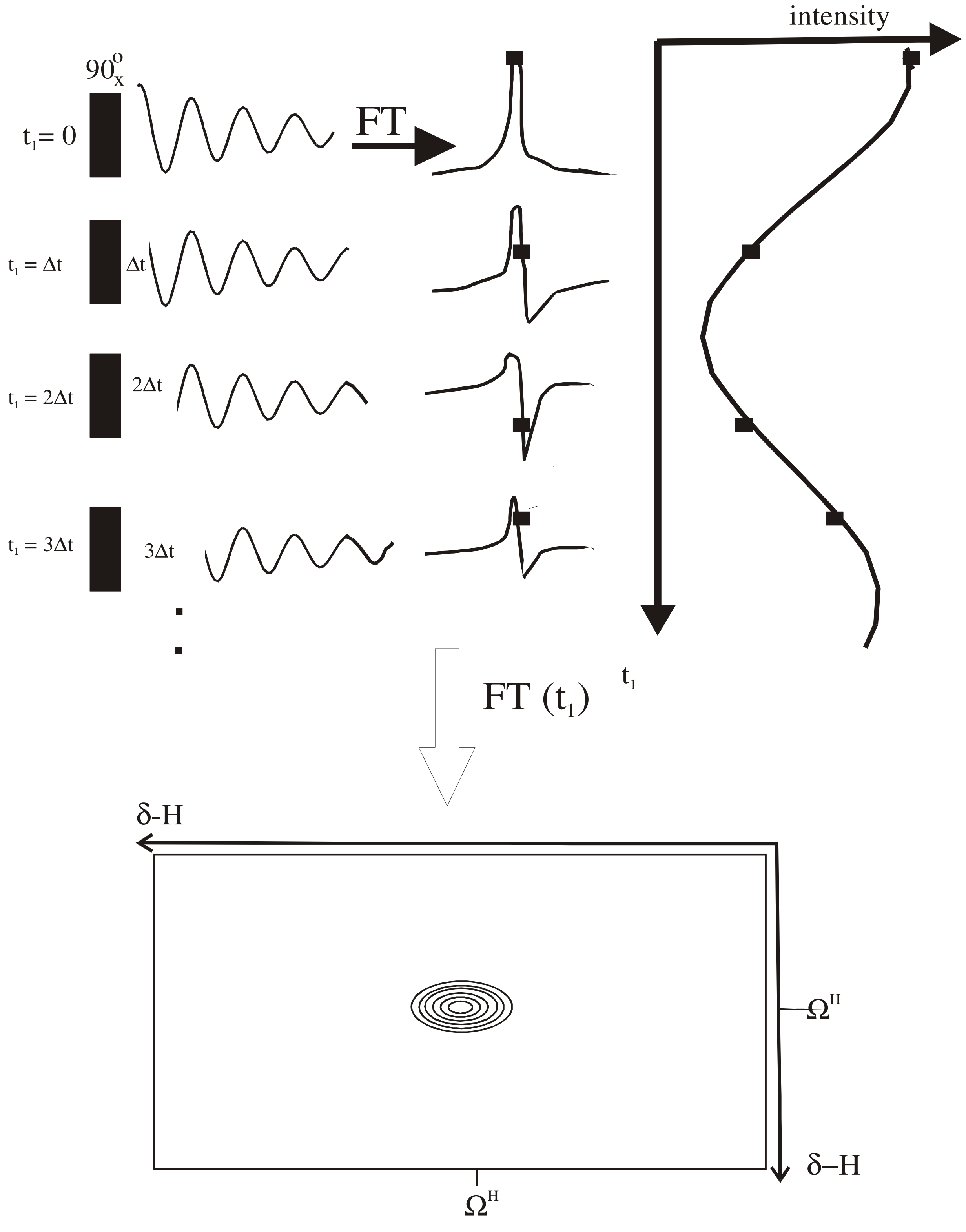
$-M_0\, I_y \cos(\Omega t_1) \cos(\Omega t_2)$

two time dependent factors give two frequencies after FT

Repeating the experiment with incrementing the delay time t_1 in a systematic manner results in a series of FID's whose phase and intensities will depend on the length of t_1. Fourier transform of those FID's will result in a series of spectra which will represent FID's oscillating with Ω and decaying with T_2^* as a function of t_1.

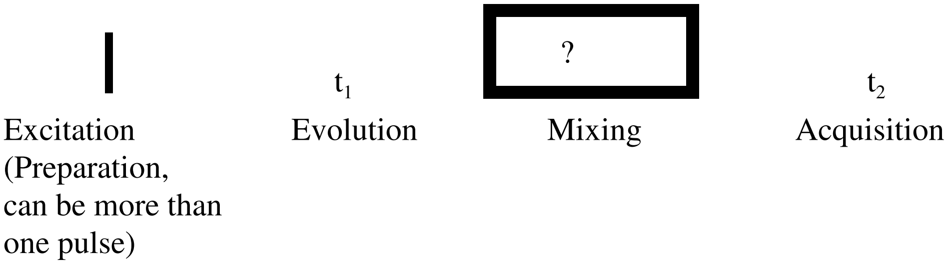
A second Fourier transform (this time along t_1) will give a second frequency axis and a peak at $f_1 = \Omega^H / f_2 = \Omega^H$.

While this is a two dimensional spectrum with two frequency axes, no additional information is present in such a spectrum as the active frequencies in both dimensions are identical ($f_1 = f_2$).



90^o_x
$t_1 = 0$
FT
intensity
$t_1 = \Delta t$
Δt
$t_1 = 2\Delta t$
$2\Delta t$
$t_1 = 3\Delta t$
$3\Delta t$
t_1
FT (t_1)
δ-H
Ω^H
δ–H
Ω^H

For a useful experiment we need an additional step to make the first evolution frequency f_1 different from the detection frequency (f_2). =>some kind of magnetization transfer has to occur between t_1 and t_2 (mixing). That results in the prototype 2D experiment:



2 Two dimensional INEPT experiments

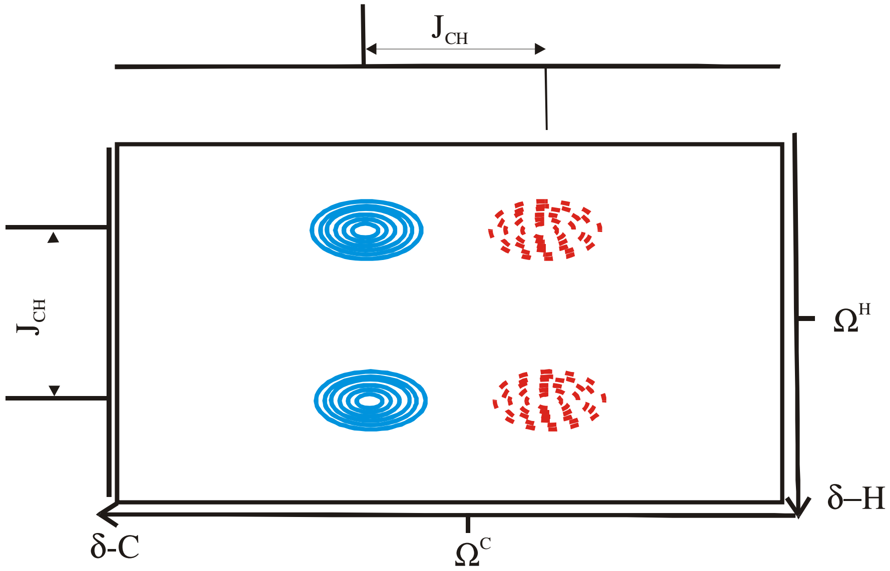
The INEPT sequence transfers magnetization from ^{1}H to a hetero nucleus. If an INEPT-type transfer occurs between t_1 and the acquisition time t_2 , evolution will take place with $f1 = \Omega^H$ during t_1 and with $f_2 = \Omega^C$ during t_2 (acquisition). The two frequency axes will therefore differ.

All what is needed is to modify INEPT experiment by adding evolution time t_1 between excitation of proton magnetization and the transfer step:

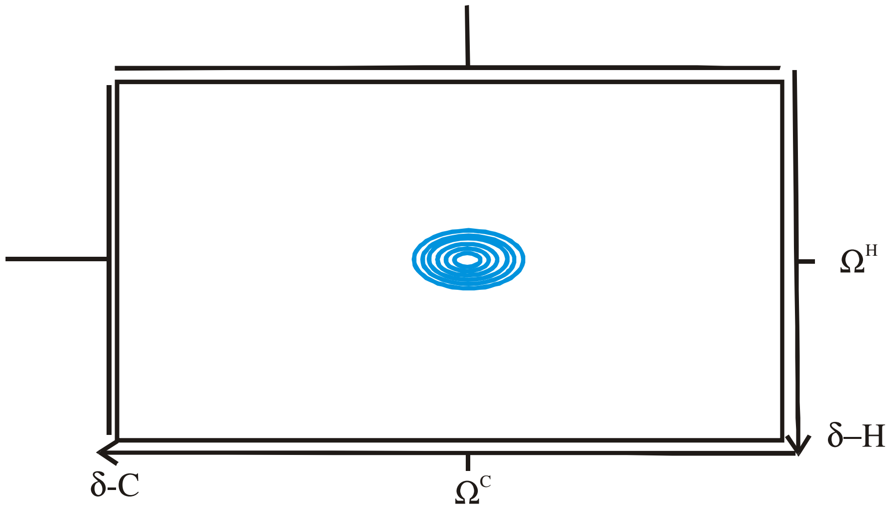
^{1}H:	$(90°)_x$	t_1	$\tau/2$	(180°)	$\tau/2$	$(90°)_y$	
^{13}C:				(180°)		$(90°)_x$	Acquisition (t_2)
	Excitation	Evolution	Mixing				Detection

The above sequence will yield doublets in f_1 and anti phase doublets in f2. Adding a 180° ^{13}C pulse in the center of t_1 and refocusing before detection will allow for a fully decoupled ^{1}H/^{13}C correlation spectrum:

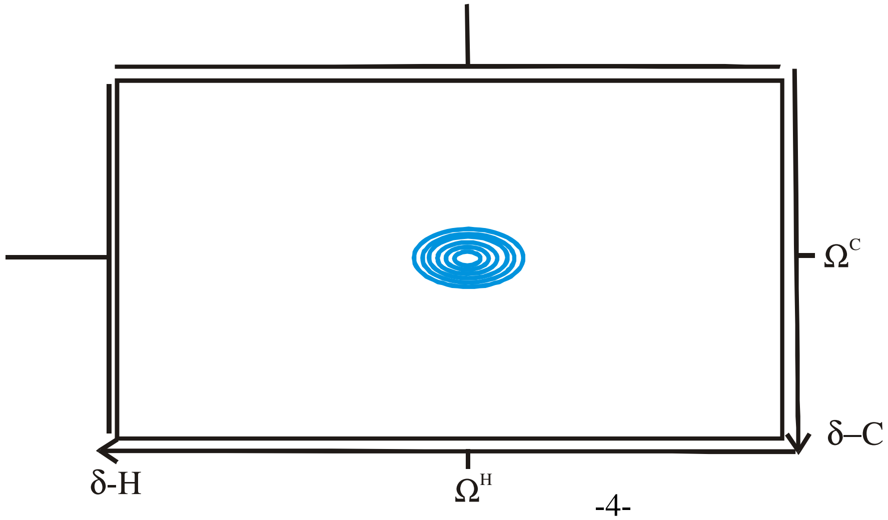
^{1}H:	$(90°)_x$	$t_1/2$		$t_1/2$	$\tau/2$	(180°)	$\tau/2$	$(90°)_y$		(180°)		__ CPD__
			(180°)			(180°)		$(90°)_x$	$\Delta/2$	(180°)	$\Delta/2$	Acquisition



Basic 2D INEPT without decoupling



2D INEPT with decoupling during both t1 and acquisition



Inverse INEPT or HSQC: Proton is the observed nucleus which is by convention plotted in the horizontal

3 Relative sensitivities of heteronuclear Experiments:

$$ {}^{\text{Signal}}/_{\text{Noise}} \sim N\cdot\sqrt{ns}\cdot Q\cdot \underbrace{\text{Polarization}}_{\gamma B_0/kT}\cdot \underbrace{\mu_{\text{observed}}}_{\gamma\sqrt{I(I+1)}}\cdot \underbrace{\text{induction}}_{\sim\sqrt{\omega_0}\ \sim\sqrt{\gamma B_0}}\cdot T_2^*/T_1\cdot \text{Efficiency of experiment} $$

N: number of spins
- concentration
- isotope abundance
- tube diameter
- length of coil

ns: number of accumulations

Q: Quality factor of probe
- coil geometry
- fill factor (Microprobe)
- Cryogenic Probe

Polarization: Excess of excited spins in lower level $\sim \gamma B_0/kT$

μ_{observed} depends on nature of observed isotope

T_2^*: determines line width

T_1: determines repetition time

induction: actual voltage induced in coil

Efficiency: how much of the total magnetitization can be transferred, loss due to T_2

S/N $\sim B^{3/2}$ stronger magnets help more than proportional

$\sim 1/T$ but note: T_2 will also decrease with lower T, also solvents will freeze

$\sim ns^{1/2}$, $\sim N$: half the concentration will require four times as long for dame S/N

$\sim \gamma^{5/2}$: proton by far most sensitive nucleus

Case of INEPT: protons are initially excited, but carbon detected: $\gamma_{\text{excited}} \neq \gamma_{\text{observed}}$

$S/N \sim \gamma_{\text{excited}} \cdot (\gamma_{\text{observed}})^{3/2}$

Nature of observed isotope has larger influence on S/N than Boltzmann Distribution of excited magnetization:

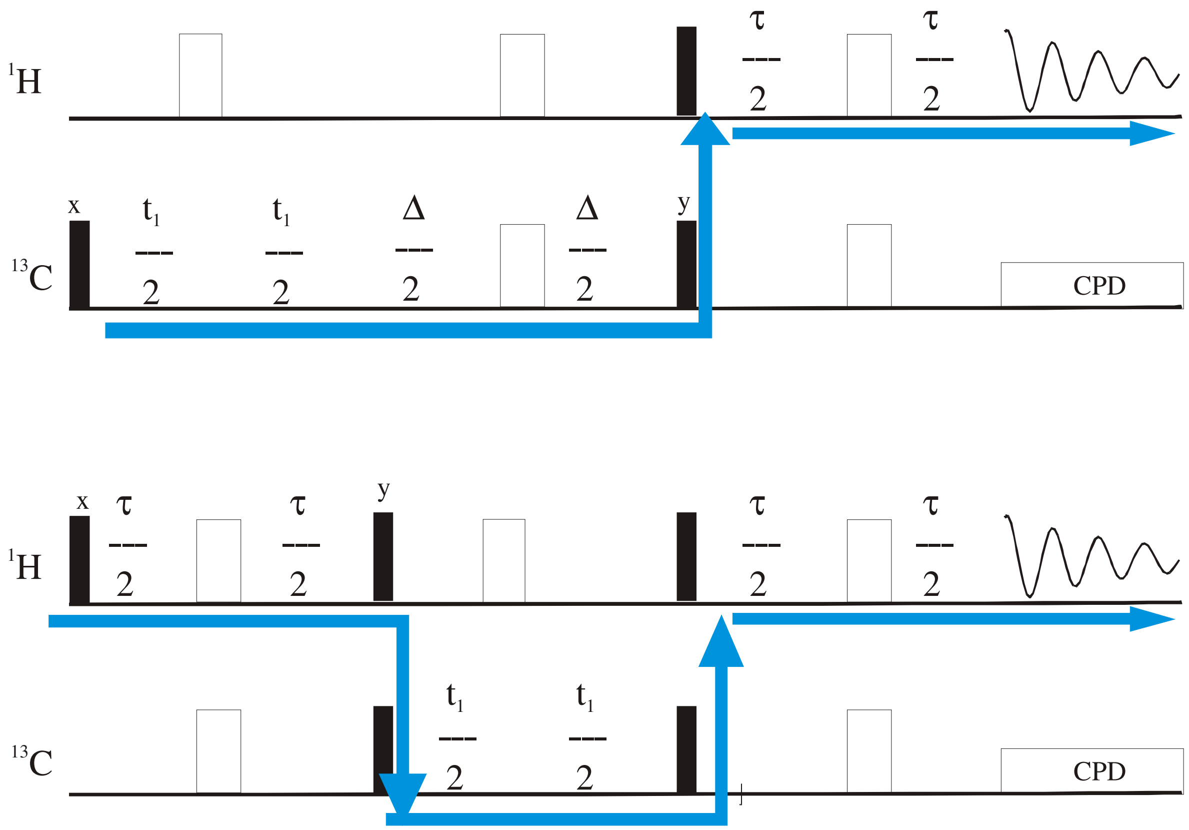
H/X- experiment	nucleus excited	nucleus detected	rel S/N	
			X = ^{13}C	X = ^{15}N
1 pulse	X	X	1	1
1 pulse + NOE	X (+½ H)	X	3	-4
INEPT, DEPT	H	X	4	10
inverse INEPT	X	H	8	30
X filtered ^{1}H, HSQC	H	H	32	300

4 Inverse detection

It is actually more sensitive to perform the 2D INEPT in the reverse manner: Excite carbon, evolve ^{13}C in indirect dimension, INEPT transfer from ^{13}C to ^{1}H, and detect proton (with ^{13}C decoupling). This is the same experiment as above, just with ^{1}H and ^{13}C interchanged and is often referred to as reverse INEPT.

By convention, the indirect dimension is always labeled t_1 and f_1 and is plotted vertically, and the detected dimension t_2 and f_2 is plotted horizontally.

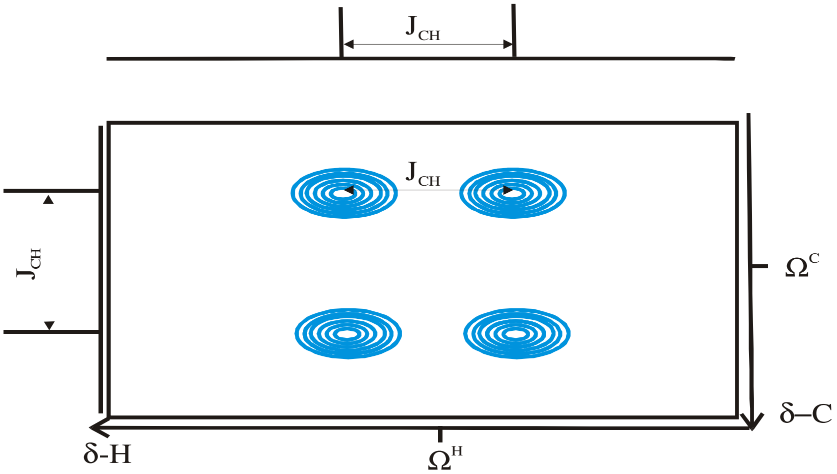
Even better sensitivity can be achieved by making ^{1}H both the excited and observed nucleus: One starts by exciting the protons, transfers the magnetization to the ^{13}C via INEPT, evolves the ^{13}C magnetization in the indirect dimension, transfers back to ^{1}H, refocuses and eventually detects ^{1}H. That is performed in the HSQC experiment (**H**eteronuclear **S**ingle **Q**uantum **C**orrelation) which is the standard experiment for C-H correlations through one bond.

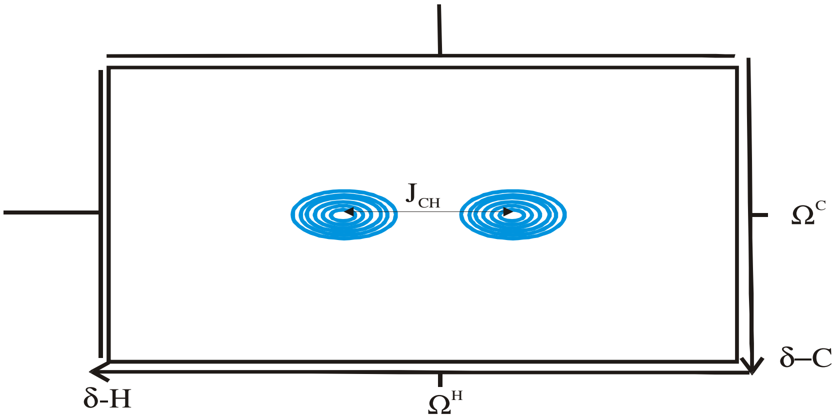


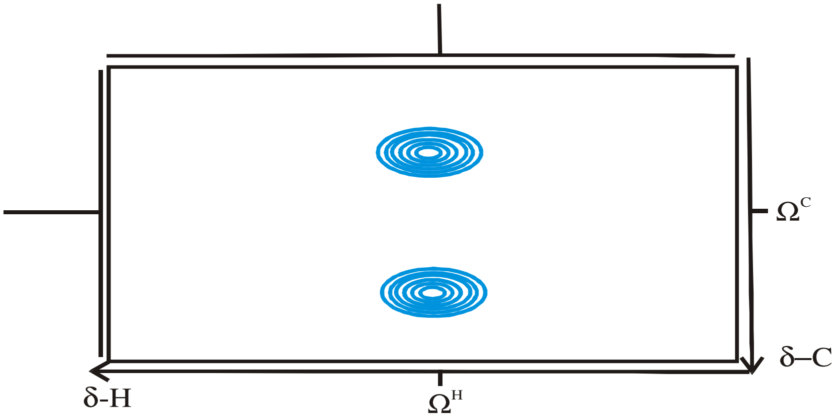
Inverse INEPT (top) and HSQC experiment (bottom) with the flow of magnetization indicated by arrows

The HSQC experiment can be performed with or without decoupling in either dimension by leaving out the proton π pulse during t_1 or the CPD decoupling during t_2.

That allows C-H couplings to be observed in 2D spectrum.

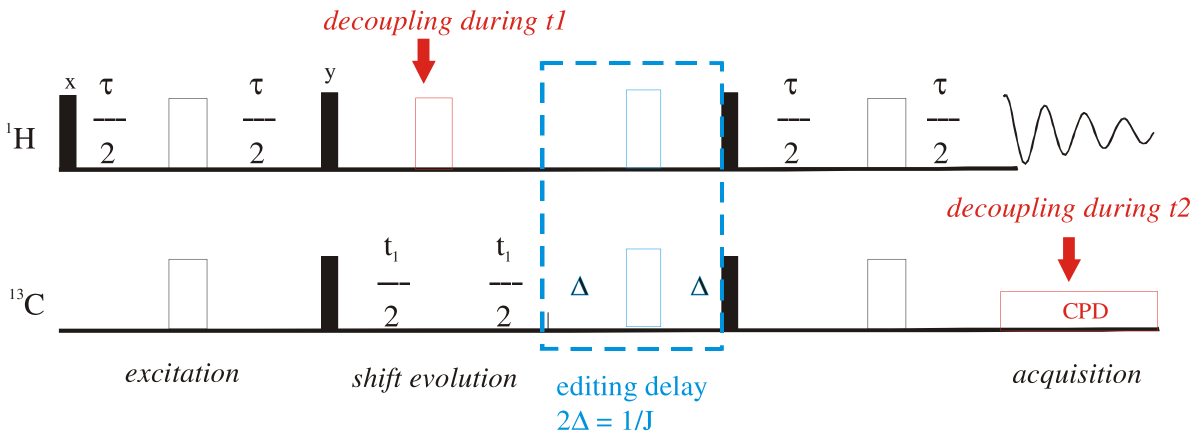


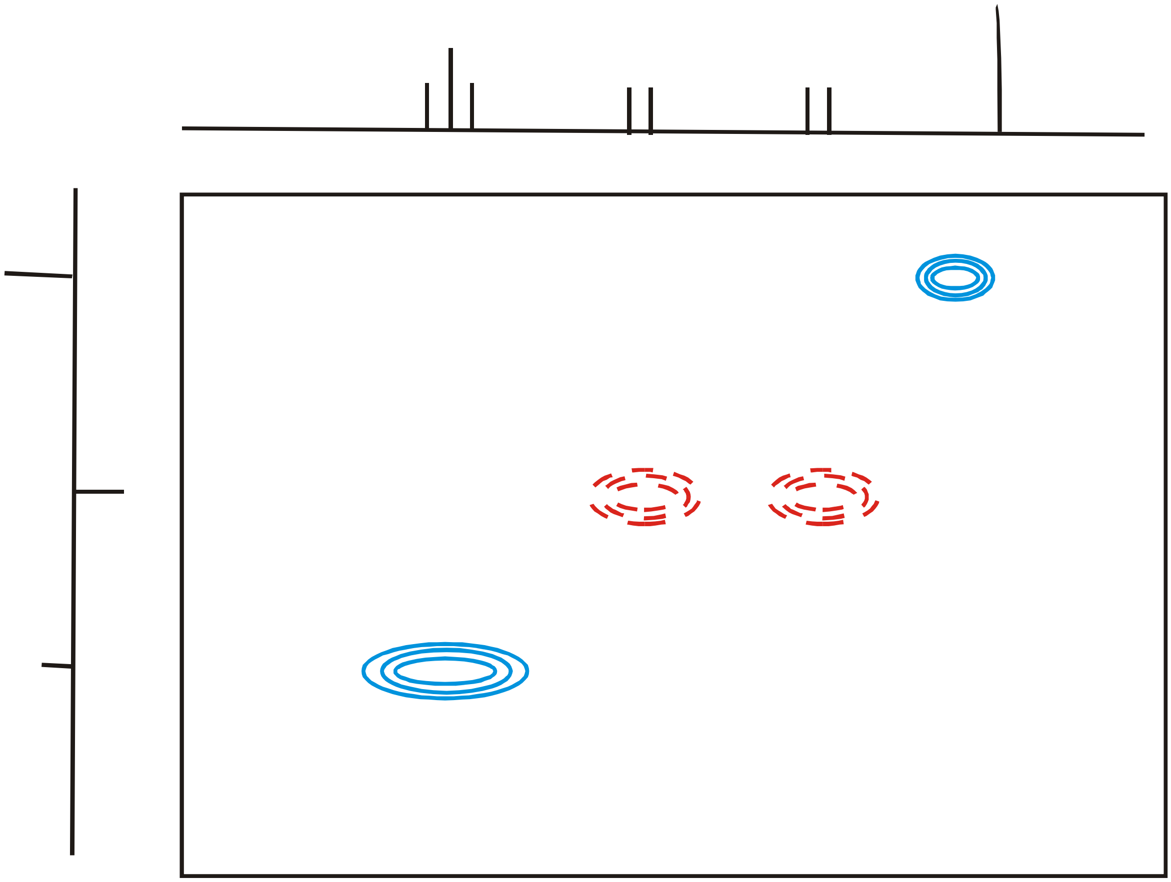




4.1 Edited HSQC Experiment:

As magnitude of coupling not needed most of the times, information about coupling partners can be obtained by performing it in an edited manner by adding a refocusing delay after t_1. CH and CH_3 groups will appear positive, CH_2 groups negative. Peaks of opposite sign are typically plotted in different color or linestyle.





4.2 HMQC Experiment

Alternative to HSQC: HMQC experiment (**H**eteronuclear **M**ultiple **Q**uantum **C**orrelation)

HMQC can give the same spectrum as HSQC, but is not as easy to understand on the basis of the vector picture

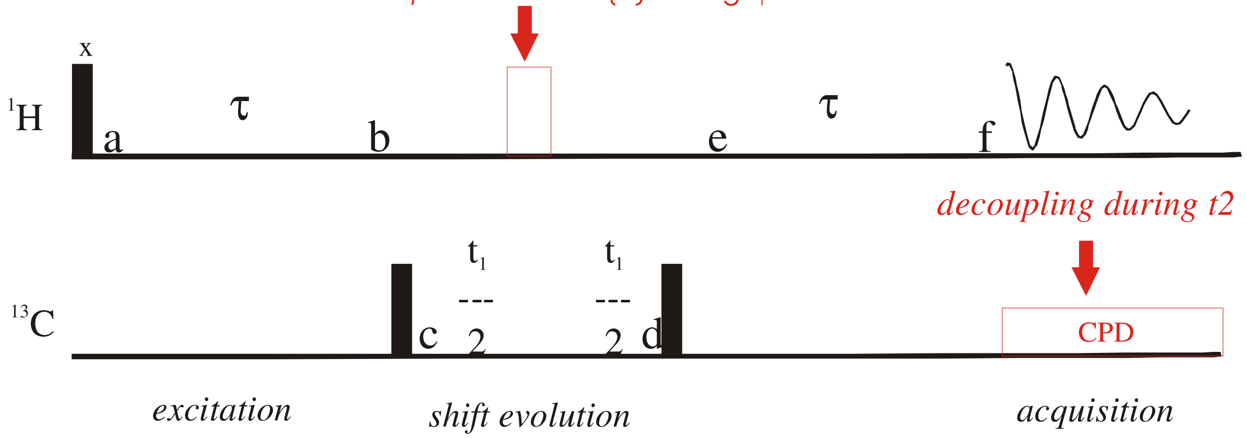
Advantages: simpler (and hence more robust) pulse sequence (less pulses)

Disatvantages: ^{12}C-H are not as easily suppressed

^{1}H/^{1}H coupling active during t_1 and will therefore give broader lines and harder to get phase sensitive spectra

It will be the experiment of choice for long range correlations.

no C-H coupling active during t_1
180 pulse refocuses proton chemical shift Ω of I_x^H component of Double Quantum operator and Ω(H) during t_1



decoupling during t2

a) $-I_y^H$ b) $I_x^H I_z^C$ c) $-I_x^H I_y^C$ d) $-I_x^H I_y^C \cos(\Omega t_1) + I_x^H I_x^C \sin(\Omega t_1)$ e) $I_x^H I_z^C \cos()$ f) $I_y^H \cos(\Omega t_1)$

5 Experimental Considerations of 2D NMR

5.1 Data size and acquisition time:

For a typical 1D spectrum 32,000 data points are taken (aq ≈2 s). A two dimensional spectrum of this size would consist of 32,000 x 32,000 points, and with 4 bytes/point take **~ *16 GB*** disk space and memory. Also with 16 scans each it would take ***23 d 16 h*** to acquire. Clearly we have to rethink our requirements. => We reduce td in both t_1 and t_2

Typically, FID's of 2048 points each are taken (td[f2]), and 128 - 512 experiments are performed (td[f1]). That results in file sizes of around 16 MB.

As signal to noise depends both on ns and td[t1], less scans per spectra can be done: with ns = 4 and above td settings spectra can be acquired in 30 min - 1h.

- The result of these very short acquisition times are truncated FID's. Therefore almost always sine function will have to be applied to the data prior to Fourier transform. On Bruker parameter **wdw**=qsine (squared sine function) and **ssb**. **ssb** = 1 (or 0): sine (starting at 0) **ssb** = 2: cosine (starting at 1) **ssb** > 2: shifted cosine (resolution enhancement). For HSQC, ssb(f1,f2)=2. For COSY: ssb(f1,f2)=0. For HMBC, ssb(f1)=2, ssb(f2)=0
- Also linear prediction can add points to data, in particular in f1 dimension: **me_mod** = lpfc, ncoef > max. number of peaks in one slice, lpbin ≈ 2·td
- Also doubling si sometimes improves quality

Setting ns versus td[1]:

- increasing ns will only increase S/N
- increasing td[1] (number of spectra taken) will increase both S/N and resolution in f1 given that aq[1] < T_2^*. This is usually the preferable solution, but in extreme cases (**aq** >= T_2^*) will not add more signal.

5.2. Selecting the desired signal - Phase cycling:

Only protons bound to ^{13}C will contribute to 2D signal at Ω^C in f_1 (1.1% of all protons). 98.9% of protons are bound to ^{12}C and will not oscillate during t_1 and hence give huge signal at $\Omega_C = 0$ Perfect HSQC will not excite ^{12}C-H, but that will never completely the case in practice.

The problem is the same as constant DC offset of signal (see Pt. 2) and can be removed by phase cycling:

$(90°)_x$ - τ/2-(180°) - τ/2 - $(90°)_y$ (180°) $(90°)_x$ - τ/2- (180°) - τ/2 -Acquisition
(180°) $(90°)_x$ - $t_1/2$ $t_1/2$ - $(90°)_\phi$ (180°) -- CPD–
ϕ = x,-x Add, subtract

^{12}C-H	$-I_y^H$	$+I_y^H$	I_y^H	$-I_y^H$	$-I_z^H$	I_z^H
^{13}C-H	$-I_y^H$	$-I_x^H I_z^C$	$I_z^H I_y^C$	$-I_z^H I_y^C$	$Iy^H I_z^C$	I_x^H

Protons bound to ^{13}C will be effected by pulse on ^{13}C, ^{12}C-H will not. Switching the phase of the second ^{13}C pulse by 180° for the second scan and subtracting the spectrum from the first one will add up ^{13}C-H, but subtract ^{12}C-H:

scan	ϕ	detected magnetization	receiver
1	x	$M_0(^{13}\mathrm{C\text{-}H})\, I_x^H \cos(\Omega^C t_1) + M(^{12}\mathrm{C\text{-}H})\, I_x^H$	ADD (+x)
2	-x	$- M_0(^{13}\mathrm{C\text{-}H})\, I_x^H \cos(\Omega^C t_1) + M(^{12}\mathrm{C\text{-}H})\, I_x^H$	SUBTRACT (-x)
		$2\cdot M_0(^{13}\mathrm{C\text{-}H})\, I_x^H \cos(\Omega^C t_1) + 0$	

5.3. Signal selection/suppression within one scan

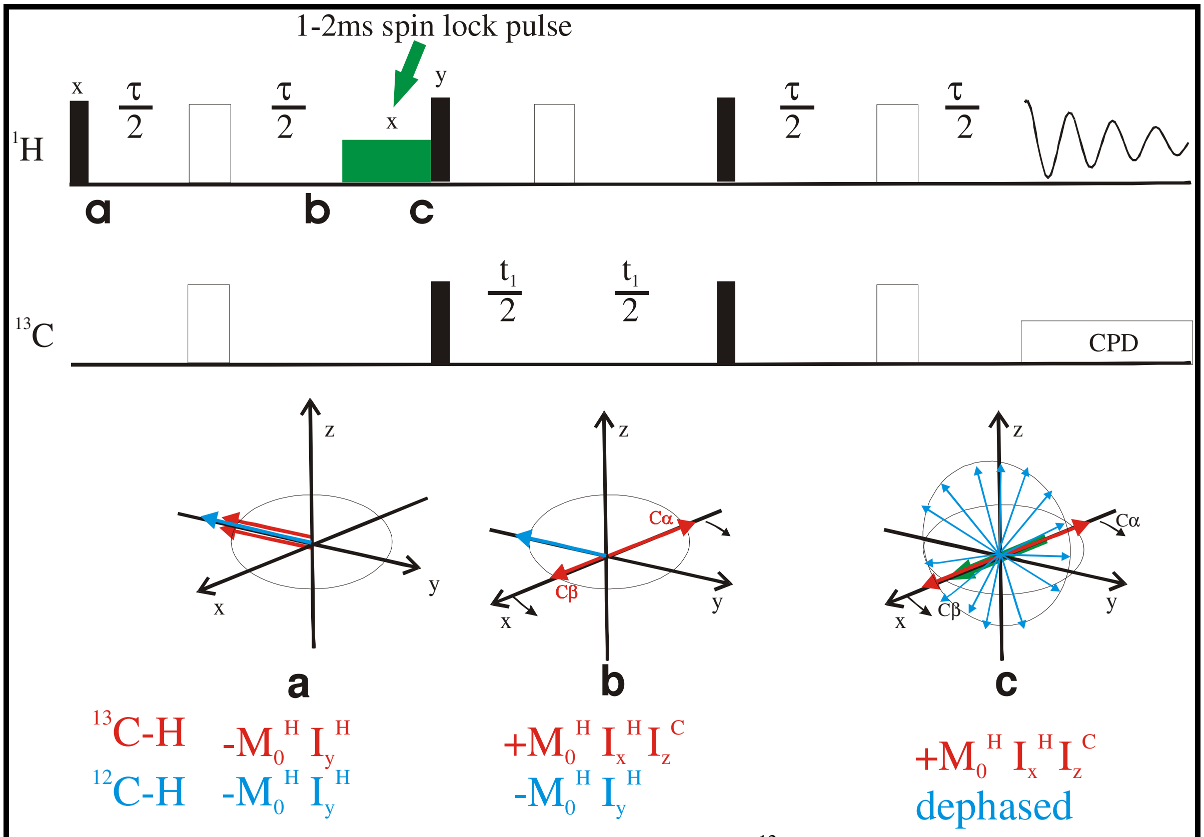
Disadvantages of phase cycling:

- several scans have to be acquired per spectrum even if sensitivity does not require it
- Incomplete subtraction due to instability will generate extra noise in the vertical direction (t_1 noise).
- Receiver gain has to be set according to full signal.

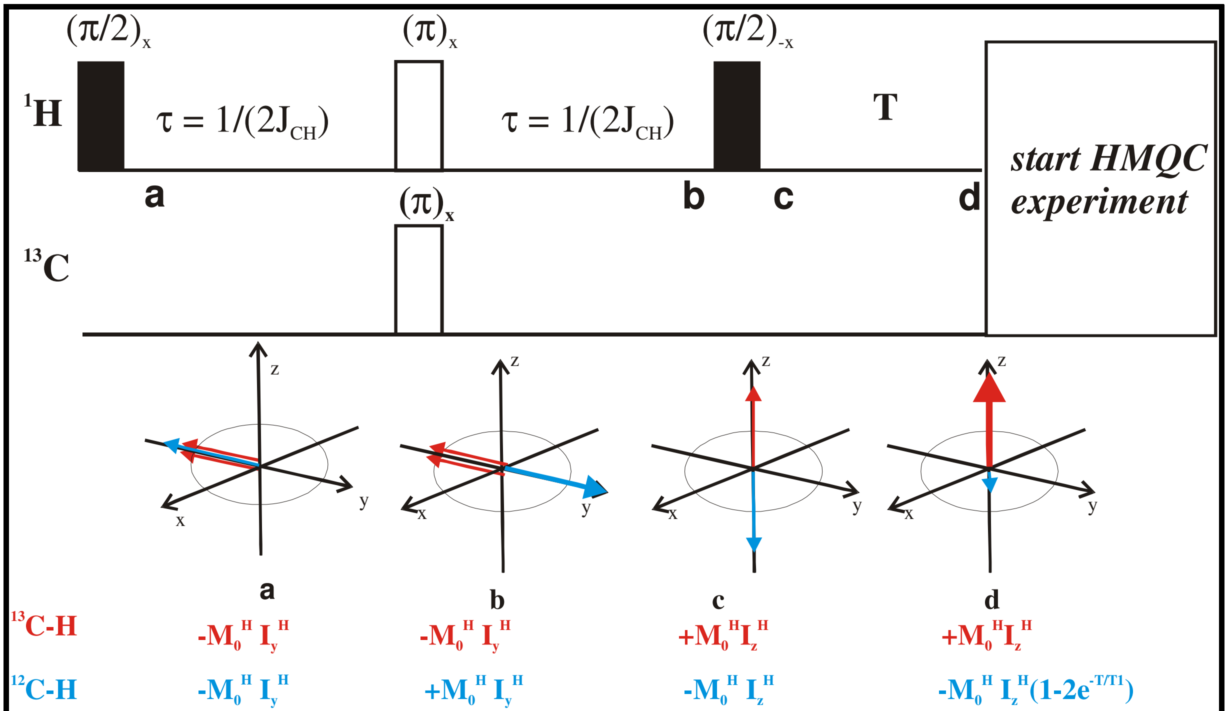
Signal selection within one scan results in much cleaner spectra.

5.3.1. Use of Purge pulses:

Example HSQC: Insert a 1-2 ms pulse along x before the INEPT transfer pulses (green pulse in figure. At this point, ^{13}C-**H** will be in antiphase along x, but ^{12}C-**H** will remain along y axis. A pulse applied parallel to a magnetization will not rotate that magnetization, but keep it in place as any dephasing due to chemical shift will be constantly reversed (spin lock). With regard to ^{13}C-**H** such a pulse will therefore have no effect. Magnetization perpendicular to such a long pulse will be rotated by 100 full rotations or more (360000° pulse). As pulses will never act perfectly the same throughout the sample, the magnetization will spread out and be dephased.



Use of a spin lock pulse in HSQC experiments to dephase ^{12}C-H magnetization



BIRD pulse for selectively inverting ^{12}C-H protons over ^{13}C-H protons. The HMQC sequence is started when $T = -\ln(½)\, T_1$ and $M_z \approx 0$

5.3.2. BIRD Pulses in HMQC

As there is no chemical shift refocusing before transfer, the above method will not work in HMQC. At the same time, ^{12}C-H will be fully exited before acquisition in HMQC experiment. The BIRD (*Bi*linear *R*otation *D*ecoupling) sequence is a common block to selectively invert signal from ^{12}C-**H** compared to ^{13}C-H (figure below). With $\tau = 1/J$ the magnetizations of ^{12}C-H and ^{13}C-H will be both in phase, but in opposite direction along y similar to the APT experiment. The $90°_{-x}$ pulse will rotate the **^{13}C-H** vector back along +z, the ^{12}C-H vector along -z. The former will be back at equilibrium. The later will be in non equilibrium, and relax back to equilibrium according to $I_z(^{12}\text{C-H}) = M_0[1-2 \cdot \exp(T/T_1)]$. If the delay T is chosen as $T = -\ln(½)\, T_1 \approx 0.69\, T_1$ then the z-magnetization of those protons will be zero.

5.3.3. Pulsed field Gradients

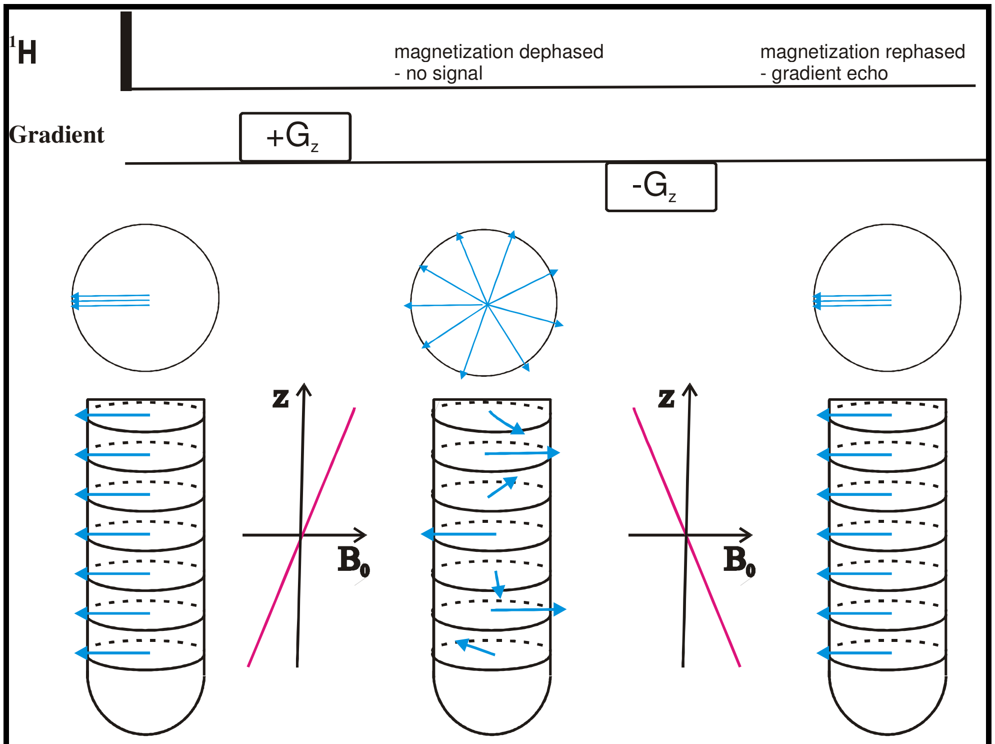
Modern spectrometers are equipped with pulsed field gradients. Like the shim coils they can produce field gradients but

- Usually only linear gradients are produced
- Gradient coils are built into probe
- Gradients are much stronger than the ones produced by shim coils
- They can be turned on for only a short time (1-2 ms) and off again for detection

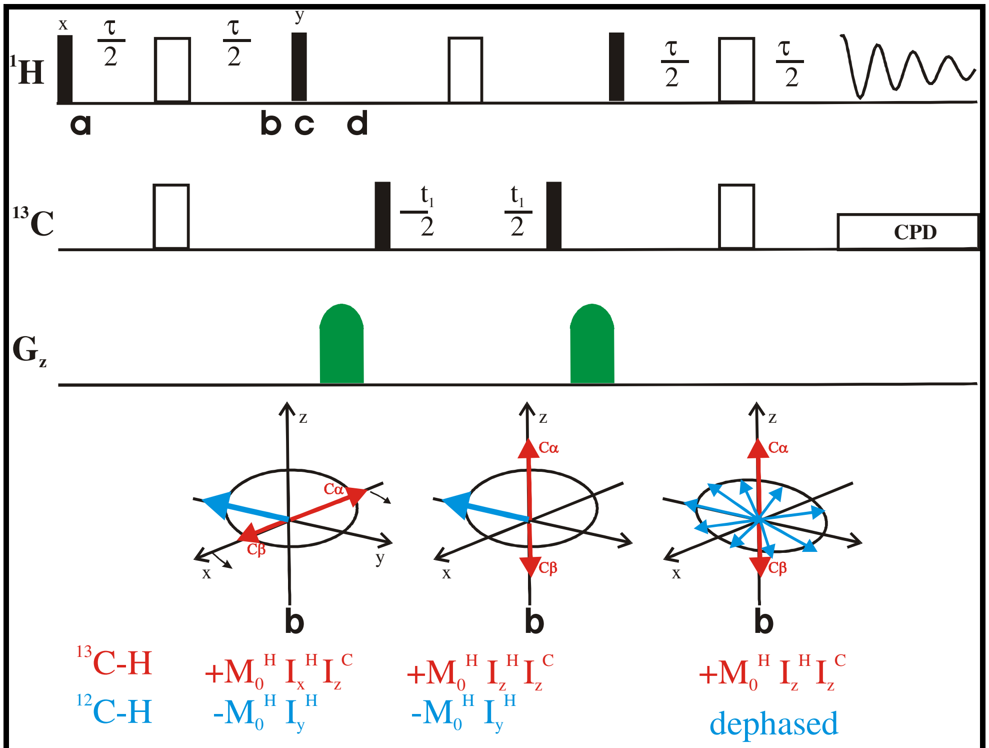
In the presence of a field gradient nuclei in different parts of the sample experience a different field. Any transversal (x,y) magnetization will dephase and cancel the macroscopic signal will be zero. Any magnetization along the z-axis will not be effected

=> Gradients can be used to dephase unwanted magnetization.

Example HSQC: After the $90°_y$ pulse at the end of the evolution time (point **c** in figure) the ^{12}C-**H** protons are along the y-axis, but the ^{13}C-**H** protons form H-C z-spin order like after selectively inverting one doublet line. A gradient applied at that point will only dephase ^{12}C-H, but not ^{13}C-H



Effect of field gradients on macroscopic magnetization



Use of purge gradients in HSQC experiment

Gradient Echos

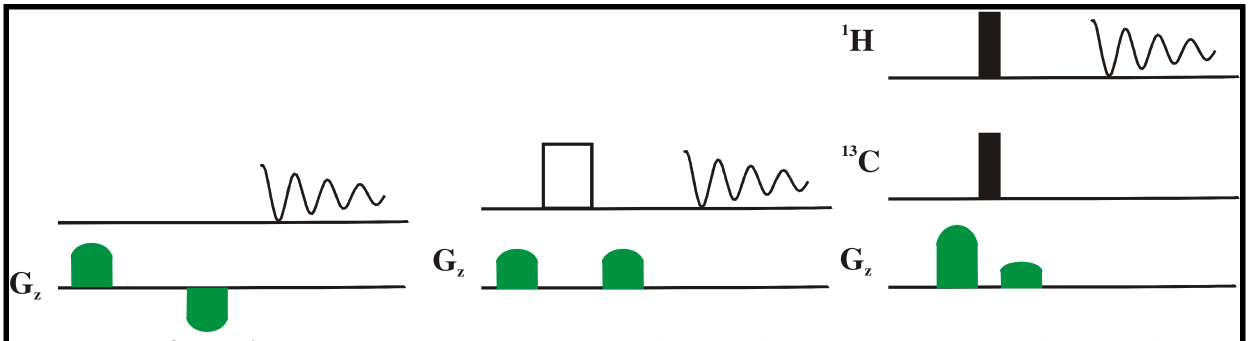
Like field inhomegeneity, the effect of field gradients is reversible. Applying a second gradient of opposite sign, but equal length and intensity will reverse the effect of the previous one (see above).

A similar effect is obtained by applying a 180° pulse between two gradients of equal length and strength

In general, the effects of gradients will cancel under the condition

$$\boxed{G_1 (\gamma_H \cdot p^1_H + \gamma_C \cdot p^1_C) + G_2 (\gamma_H \cdot p^2_H + \gamma_C \cdot p^2_C) + ... = 0}$$

with G_i the gradient strength(length x intensity), γ_i = gyromagnetic ratio of the nucleus, and p_i is the ***coherence order*** of the nucleus. It is p=0 fir I_z or $I_z^H I_z^C$, I = ±1 for I_x or I_y. 180° pulses will change the sign of p. Antiphase magnetization $I_x^C I_z^H$ has $p_C = 1$ and $p_H = 0$.



Examples of gradient echos: $G_1 = -G_2$ selects $p = i \Rightarrow p = i$, $G_1 = G_2$ selects $p = i \Rightarrow p = -i$ ($I_x \Rightarrow -I_x$ to remove imperfections of 180° pulses), and $G_1 = \gamma_C / \gamma_H \cdot G_2$ selects $p_C = i \Rightarrow p_H = i$ transformations (i.e. $I_x^C I_z^H \Rightarrow I_y^H$ in HSQC).

Gradient echos are the most efficient way of selecting desired pathways. In HSQC, the gradients are applied before and after the INEPT back transfer. For $^1H\{^{13}C\}$ a ratio of $G_1:G_2 = 4:1$ is used, for $^1H\{^{15}N\}$ $G_1:G_2 = 10:1$. A similar scheme can be employed for HMQC.

5.4. Distinguish sign of rotation in indirect dimension (quadrature detection in t_1)

In 1D NMR two detectors are employed, one detecting the y (cos), one the x component (sin).

In 2D NMR, the 90° pulse at the end of t_1 will select only x or y component of the magnetization.

For example in the HSQC the second $90°_x$ pulse on ^{13}C will only rotate magnetization along y, but not effect magnetization along x. That is equivalent to a detector measuring only the y-component of a signal. For proper detection a second scan will have to be taken with the pulse along y:

$$\ldots (90°)^C_x \quad t_1 \quad (90°)^C_{\phi} \ldots\ldots \text{ Acquisition} \qquad \phi = x,y$$

scan	ϕ	detected magnetization	receiver
1	x	$I_x^H \cos(\Omega^C t_1)$	+x
2	y	$I_x^H \sin(\Omega^C t_1)$	+x

In general, the sign of rotation in the indirect dimension can be detected by varying the phase difference of the two pulses flanking the incremented delay t_1 between 0° and 90°. Combined with the subtraction scheme a four step cycle is needed to properly perform experiment:

scan	ϕ	rec.
1	x	x
2	y	x
3	-x	-x
4	-y	-x

If sine and cos components are stored in same file one obtains a so called phase modulated signal. While distinguishing the sign of the rotation positive and negative intensities are usually not distinguished and the data are processed in magnitude mode.

If they are stored in separate files they are called magnitude modulated and phase sensitive, and a full phase correction can be performed in both dimensions. Different schemes are possible:

td1	t_1	scan	ϕ	spectrum	td1	spectrum	t_1	ϕ	t_1	ϕ	gradient	spectrum
1	0	1	x	cos±sin	1	Re cos	0	x	0	x	G2	cos + sin
	0	2	y		2	Im sin	$\Delta t/2$	y	0	y	-G2	cos - sin
2	Δt	1	x	cos±sin	3	Re cos	Δt	-x	Δt	-x	G2	cos + sin
	Δt	2	y		4	Im sin	$3\Delta t/2$	-y	Δt	-y	-G2	cos - sin
	phase mod.(QF)				States		TPPI		States-TPPI		Echo-Anti echo	

Note that in the TPPI method t_1 is incremented with half of the dwell time. On Bruker spectrometers, that i srealized to set the parameter **nd0** to twice the number of occurrences of the incremented delay d0 in the pulse sequence.

The methods also differ in the way peaks outside the spectral region are folded and the location of any remaining axial peaks (peaks with no dependence on t_1)

If gradient echos are used, quadrature detection by selecting x vs. y magnetization will not work as any transversal magnetization is spread out in the x,y plane. Either a phase modulated magnitude spectrum is obtained, or a phase sensitive spectrum can be obtained by collecting both the echo and antiecho spectra.

Magnitude spectra: no need for phase correction (easier to process), experiment may not need all refocusing delays. But: Broader lines, cannot distinguish positive and negative signals. DPX300: regular COSY, HMBC

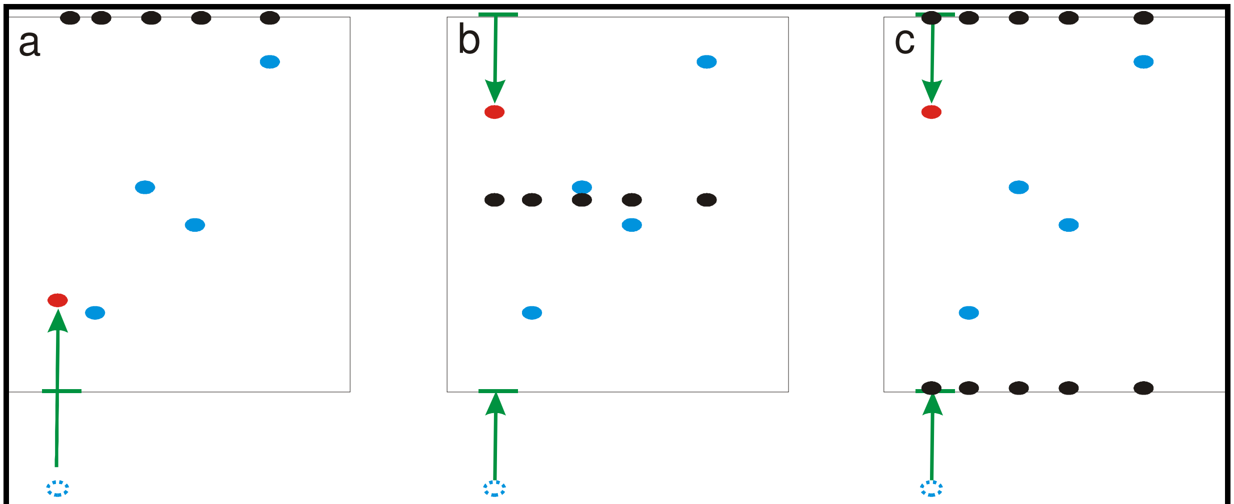
Phase sensitive spectra: Often more demanding processing, but sharper lines, distinguish positive and negative lines. On DPX-300: HSQC, NOESY, PE-COSY

5.5. Dwell time and folding

The increment t_1 (Δt) is the dwell time in the indirect dimension and determines the spectral width in f_1: $\Delta t = dw(1) = 1/(swh[1]\cdot 2)$

As there is no frequency filter in the indirect f1-dimension, all exited peaks outside the window will show up but at the wrong frequency. Watch out with standard HSQC parameters on 300: The default window does not include aldehyde carbons.

The location of peaks outside the spectral window will depend on the quadrature scheme used: In TPPI and States-TPPI they will fold back symmetrically on the same side, in States they will alias on the opposite end of the spectrum:



Location of folded/aliased (●) signals and axial peaks (●): a) TPPI b) States c) States-TPPI. Regular peaks are denoted ●

6. Structure determination using 2D NMR experiments:

$^{1}H\{^{13}C\}$ HSQC

- allows for measurement of ^{13}C (or X) chemical shift with much higher sensitivity than 1D ^{13}C experiment
- allows to connect the protons with their carbon atoms and obtain CH fragments
- identify and connect pairs of diastereotopic protons
- separate overlapping signals and account for all CH_X groups in molecule (do in conjunction with integrations in ^{1}H spectrum)
- identify OH and NH as they will NOT give cross peaks

Connecting CH fragments with $^{1}H/^{1}H$ COSY:

HSQC will not give any connectivity information between the fragments. An experiment utilizing $^{3}J_{HH}$ couplings between neighbored CH (or more general any XH-YH) fragments is the H/H-COSY experiment.

The basic COSY experiment is the simplest 2D experiment conceivable as it consists of two 90° pulses seperated by an incremented delay t_1:

$$(\pi/2)_x - t_1 - (\pi/2)_{y,-y} - \text{acquisition } (t_2)\ (x,-x)$$

While this looks much simpler than the 2D INEPT of HSQC experiments the proper theoretical treatment of the COSY experiment is more complex and will be postponed.

There are two reasons why the experiment is not done as a direct analogue to the INEPT experiment:

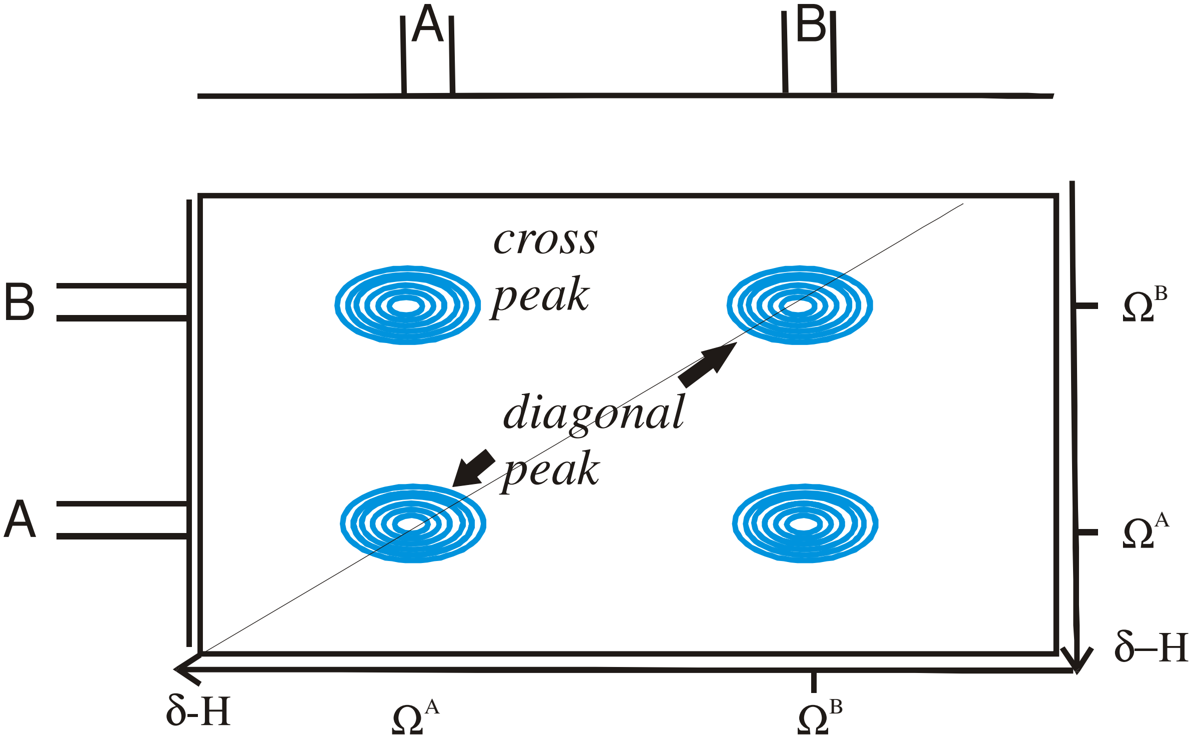
- as the active coupling is between equal nuclei, it can not be decoupled (or refocused) during shift evolution as every pulse is applied to both coupling partners
  => coupled spectrum with active coupling in anti phase
- the relative range of $^3J_{HH}$ is much larger (2-14 Hz) than for $^1J_{CH}$ (127-150 Hz) and thus no optimal mixing time τ exists

For $t_1 = 0$ no signal is observed (after phase cycling). The signal is therefore an exponentially decaying cosine in the acquisition dimension, but a decaying sine function ($\sin(\pi J_{HH}\, t_1)$) in the indirect dimension.

The resulting spectrum has a proton axis in each dimension and features two types of peaks:

$\Omega(f_1) = \Omega(f_2)$ => diagonal peak

$\Omega(f_1) \neq \Omega(f_2)$ => cross peaks



Diagonal peaks carry no additional information compared to the 1D spectrum (like in our introductory 2D [π/2 t_1 acquisition] experiment

Cross peaks occur for each pair of protons coupled by a $^{2\text{ or }3}J_{HH}$ coupling (or more general:by any coupling of significance). They always occur in pairs for a -> b and b -> a, which are arranged symmetric about the diagonal.

- Analysis of basic COSY simply searches for cross peaks connecting neighbored protons coupled by $^3J_{HH}$ ... *but watch out for the following:*
- be aware of pairs of diastereotopic protons coupled by $^2J_{HH}$. Those are normally very strong, but don't carry connectivity information. You can identify them from HSQC spectrum
- in cases of dihedral H-C-C-H angles close to 90° the $^3J_{HH}$ coupling may be very small and the cross peak very weak or not observed
- in some cases long range $^4J_{HH}$ and $^5J_{HH}$ may be large enough to result in weak cross peaks
- If peaks are very close in chemical shift, the cross peak may overlap with diagonal

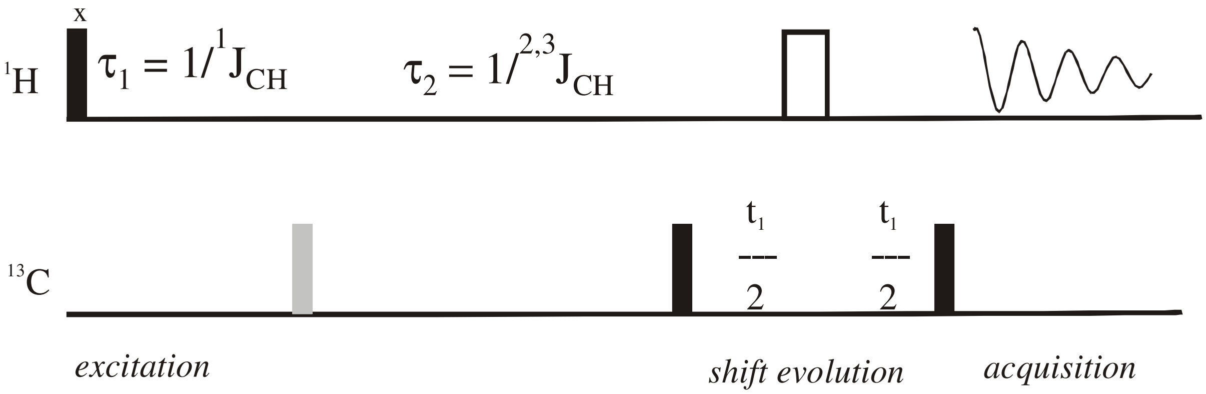
Connecting across tertiary carbons: HMBC

In addition to 3 bond H-H couplings long rang two and three bond C-H couplings can be utilized to establish connectivity along **H**-C-**C** and **H**-C-C-**C**. This is particularly useful to identify tertiary carbons and connect CH fragments separated by non protonated fragments.

The experiment is derived from the HMQC experiment employing a delay time τ optimized for two- and three bond C-H couplings ($^{2,3}J_{CH} \approx$ 4-12 Hz, τ = 50 - 70 ms). The second refocusing delay is normally omitted and the experiment recorded without ^{13}C decoupling during acquisition and non phase sensitive (magnitude display).

Often an additional 90° ^{13}C pulse is employed (grey in the figure) to suppress one bond (HSQC-type) correlation peaks.

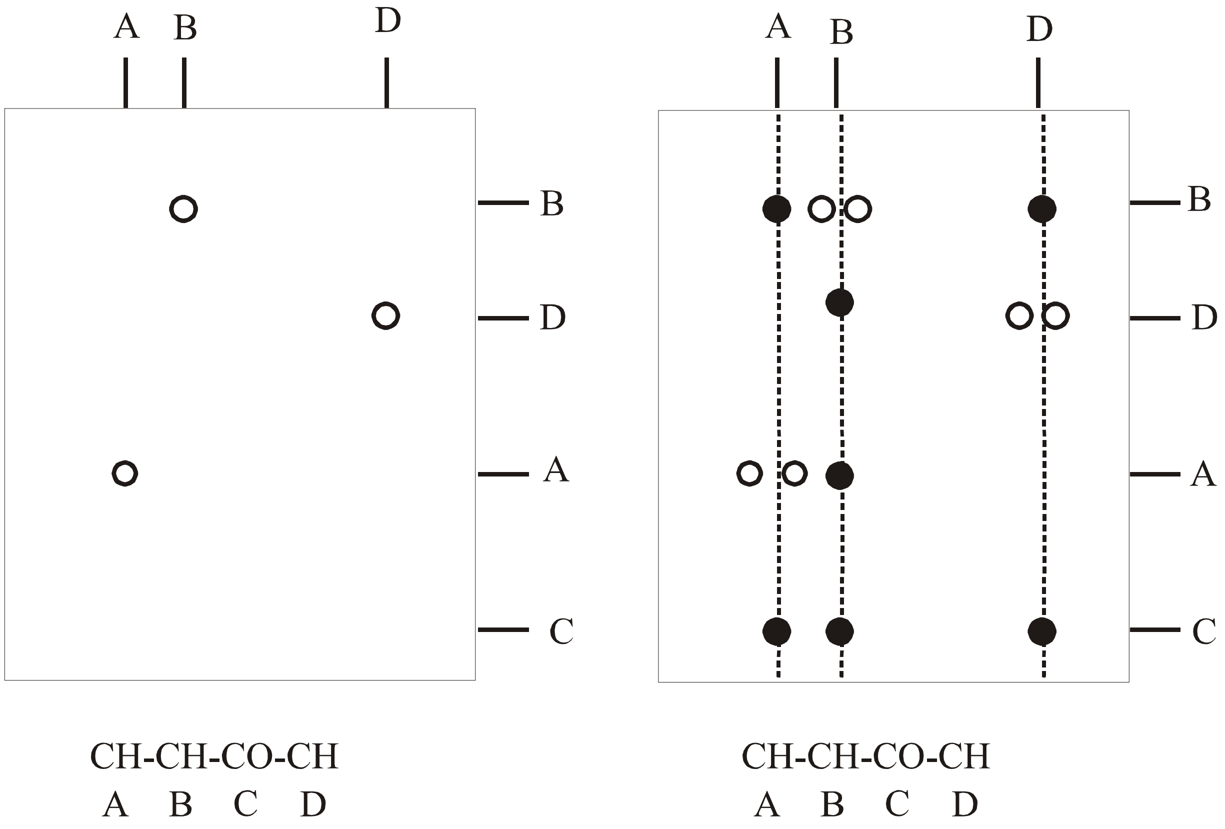
The 2D spectrum shows crosspeaks between a proton and carbon shifts separated by two and three bonds.



Analysis:

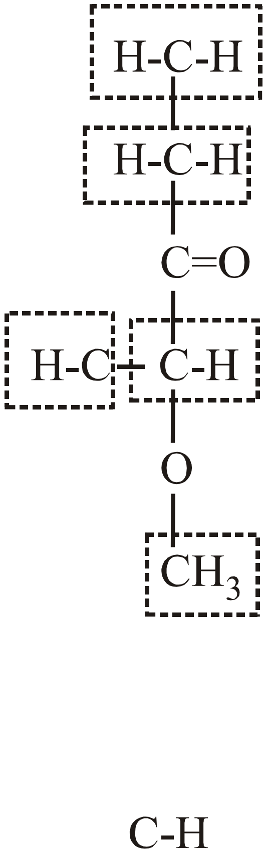
- always check with HSQC experiment to identify residual one bond correlations. These will show up as weak doublets.
- The experiment can be used to assign tertiary carbons (C=O, >C< from neighboring C-H groups)

- Allows to make connections across non protonated atoms (C=O, >C<, -O-, >N- etc.). This can be done by direct correlation (**H**-C-X-**C**H) or connecting two CH groups to a common C atom (**H**-C-**C**O-O-C-**H**)
- also NH and OH peaks can give cross peaks (***H***-N-***C*** or ***H***-O-***C***)
- Intensity of cross peak will depend on value of J_{CH}, so some three bond interactions may be weak or missing if dihedral angle (H-C-X-C) is close to 90°
- While very powerful the fact that both two- and three bond interactions show up causes many peaks in the spectrum and makes the assignment sometimes ambigious
- the proton chemical shift during mixing time τ is **NOT** refocused. Also proton-proton coupling will be active during both τ and t_1. Spectrum is not phasable and usually displayed in magnitude mode.

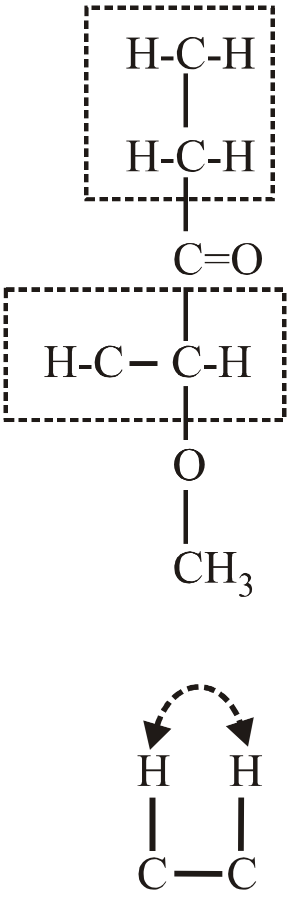


HSQC **HMBC**

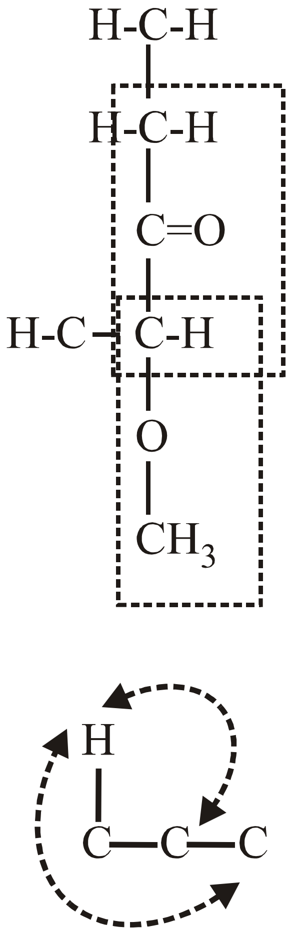
Schematic HSQC and HMBC spectra. Note that HSQC peaks will show up as weak doublets in the HMBC spectrum as no C-H decoupling is employed during t_2.



HSQC



COSY



HMBC

Most connectivity problems will be solved by a combination of HSQC, COSY and HMBC, and analysis normally takes place in that order.

Sensitivity is usually ^{1}H > COSY > ^{1}H$^{\{13}$C} HSQC > ^{1}H{^{13}C} HMBC > 1D ^{13}C

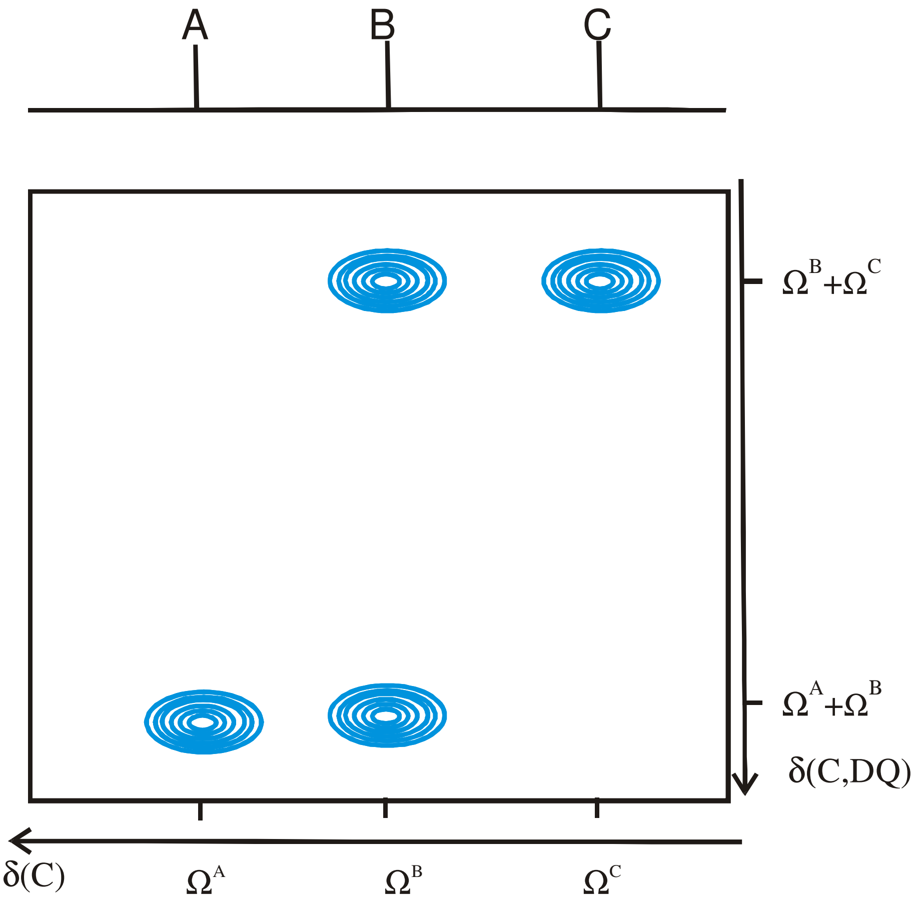
C-C correlation with INADEQUATE (**I**ncredible **N**atural **A**bundance **D**ouble **QUA**ntum **E**xperiment)

The disadvantage of HMBC is its ambiguity (***H***C-***C*** and ***H***C-X-***C*** connectivities can not be distinguished), the potential dependence of the cross peak intensity on dihedral angles and frequent overlap in the proton dimension.

One alternative is the INADEQUATE (**I**ncredible **N**atural **A**bundance **D**oubl**E** **QUA**ntum **T**ransfer **E**xperiment) which utilizes one bond ^{13}C-^{13}C couplings. This experiments selects only molecules which have two ^{13}C atoms (i.e. 0.01% of all molecules) and is thus 100 × less sensitive than a regular ^{13}C experiment.

The acquisition dimension is a regular ^{13}C spectrum, and the indirect axis displays the sum of the ^{13}C shifts of the ^{13}C-^{13}C pairs (i.e. $\delta_{CA}+\delta_{CB}$):

$$C^{A}\text{-}C^{B}\text{-}C^{C}$$



While potentially the most powerful method in elucidating C-C connectivities, it lacks sensitivity and should only attempted with samples where a ^{13}C spectrum with S/N > 10 can be obtained with one scan (which is almost never the case). In the example shown for menthol (next section) about 0.5 g of sample were used.

Relayed H-H correlation with TOCSY

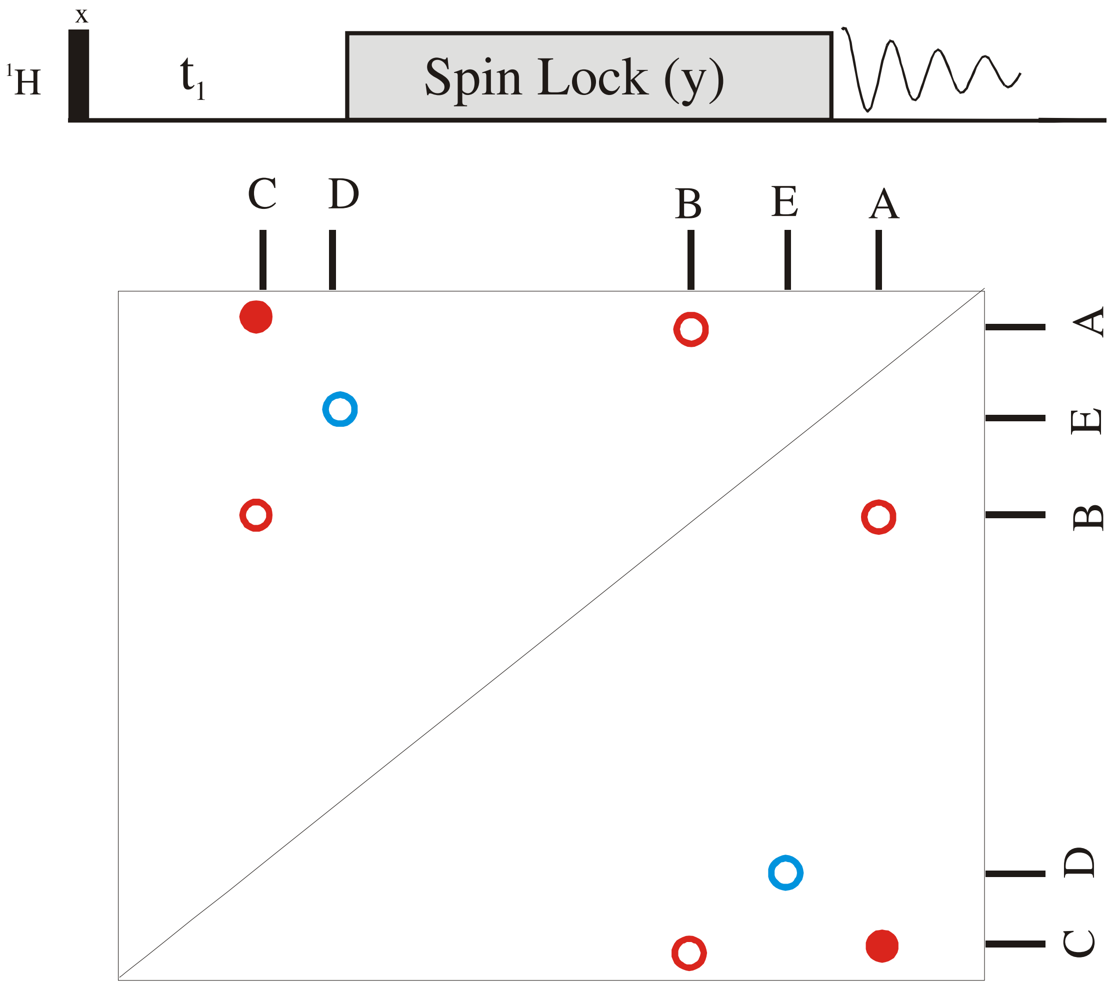
H-H correlation can be done by employing a spin lock pulse instead of the second 90° pulse in COSY. In its simplest form it is just one long pulse ($\tau_P \approx$ 10-80 ms, $\gamma B_1/(2\pi) \approx$ 10 kHz) As it is applied ‖ to the magnetization M_y it will not rotate the spins, but it will prevent chemical shift precession from occuring and keep the magnetization along the y axis (hence the name spin lock). However coupling will occur and magnetization will be transfered between coupled spins.

In practice, TOCSY experiments are performed with a series of spin lock pulses instead of one long pulse similar to the decoupling schemes.

For $\tau_{SL} \approx$ 10 ms a spectrum similar to COSY will be obtained. However, since the spin lock pulse also acts as a purge pulse, TOCSY spectra can be acquired with one scan per increment even without gradients (the parameter file **cosy.bbo** on the DRX 500 actually calls up such a TOCSY experiment)

For longer τ_{SL} (50-80 ms) relayed transfer occurs between neighbored CH groups, i.e. in a chain CH^A-CH^B-CH^C H^A will not only give a crosspeak with its neighbor H^B, but also with H^C. Ideally each proton shows crosspeaks with all members in the spin system. Therefore the name ***TO***tal ***C***orrelation ***S***pectroscop***Y***.

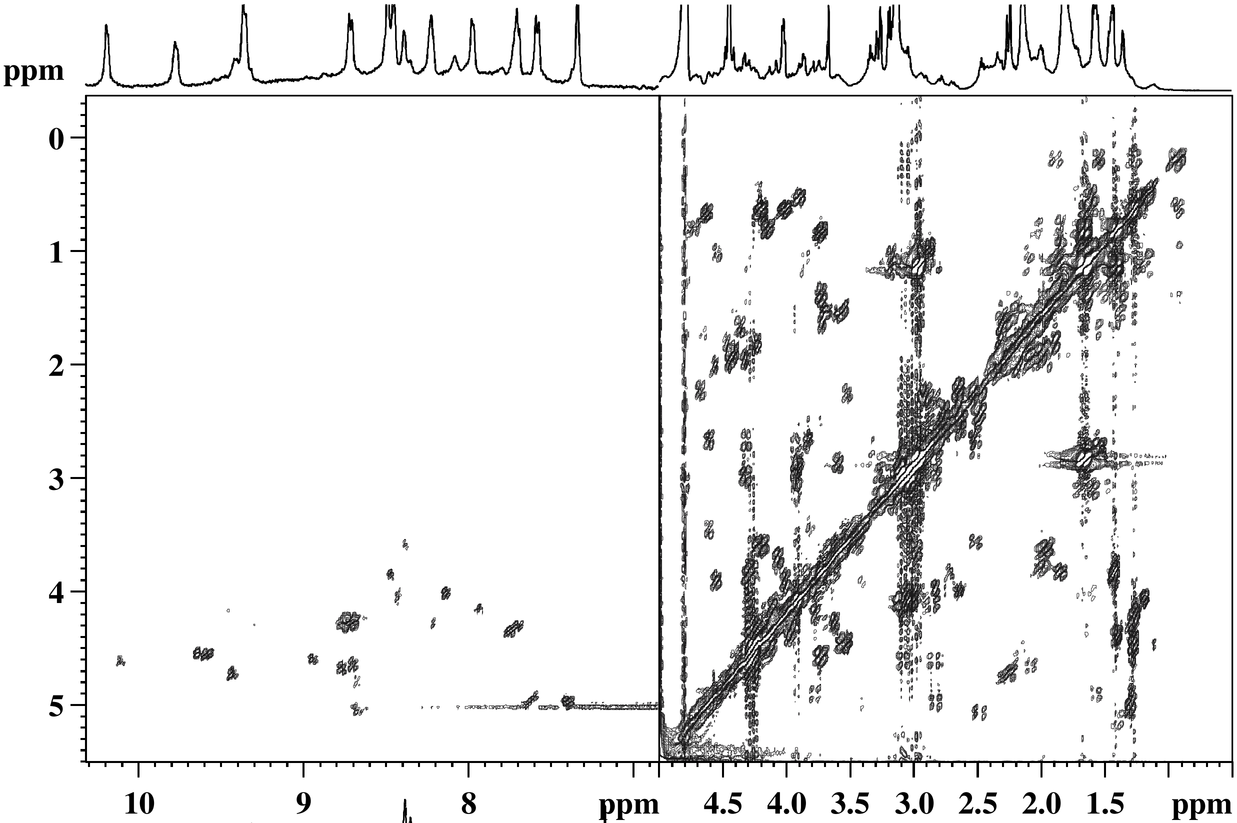
The main advantage is that in case of heavy overlap in one part of the spectrum shifts can be identified using only few separated protons (example H^N protons in polypeptides).



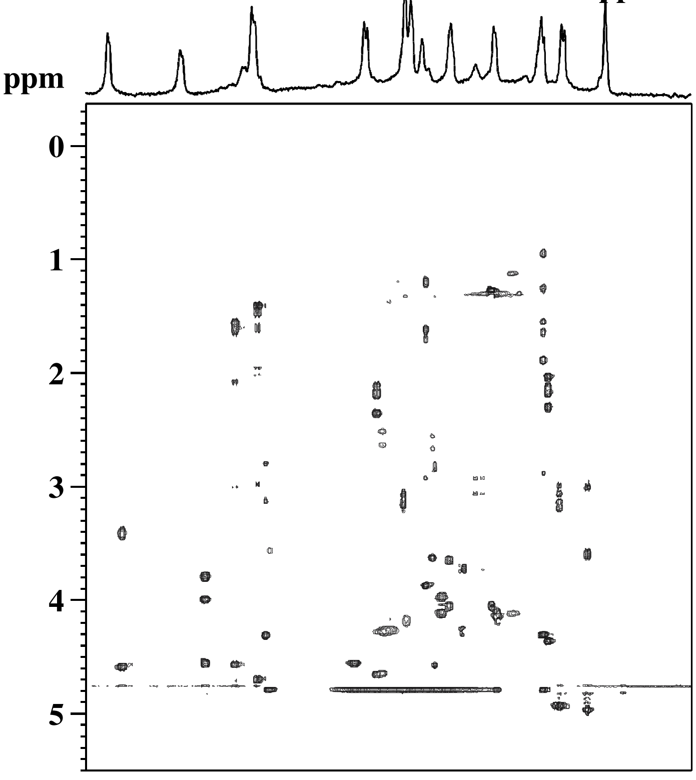
CH-CH-CH-CO-CH-CH

A B C D E

Schematic 2D H/H TOSCY spectrum. Open circles (○) indicate cross peaks also present in COSY, whereas filles circles (●) indicate relay peaks only present in TOCSY spectra with long mixing times ($\tau_{SL} \approx 50 - 80$ ms)



Top: COSY spectra of Metallothionein

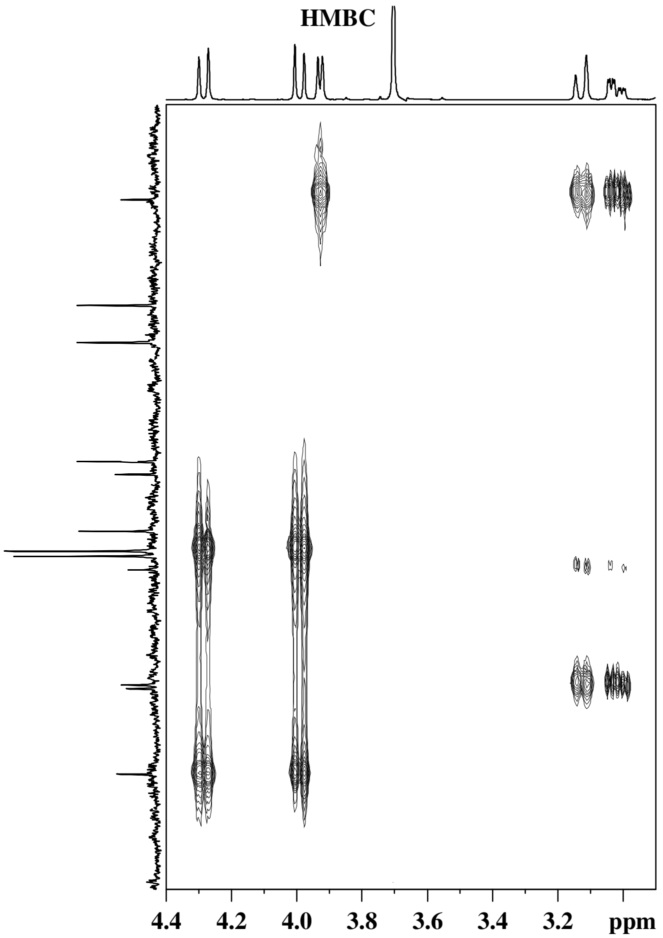


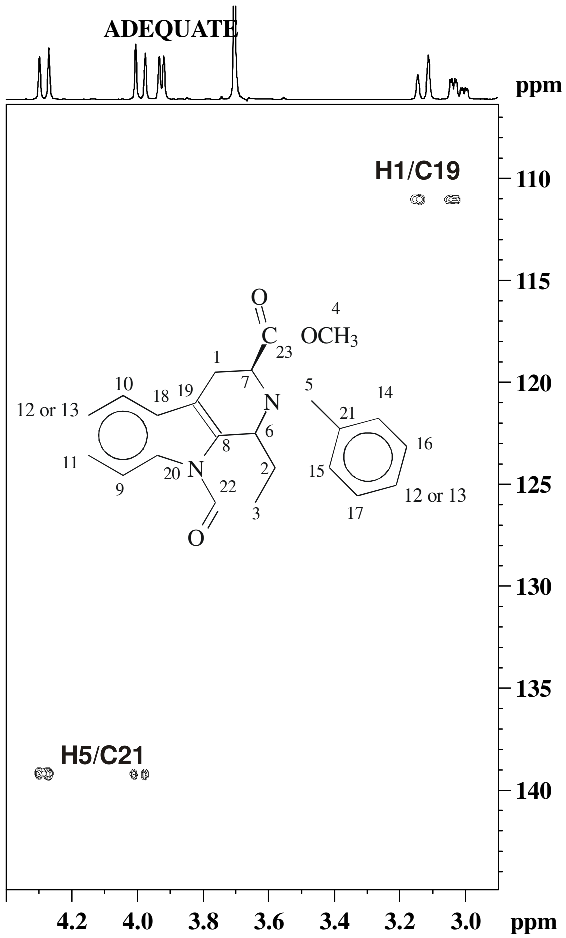
Bottom: TOCSY spectrum allows assignment of amino= acids from NH-region alone

Combining sequences:

Many experiments can be considered as combination of the above experiments using some basic mixing schemes as building block: INEPT, COSY, TOCSY, HMQC, INADEQUATE. We will later learn NOE-transfer.

HSCQ-TOCSY:	TOCY + HSQC: get a TOCSY spectrum which has the shifts of the attached carbons in the indirect dimension. Advantage of C-13 resolution in indirect dimension, but only 1% the sensitivity.
HSQC-COSY	in a similar manner one can combine HSQC and COSY
ADEQUATE:	HSQC + INADEQUATE: inverse detected version of the INADEQUATE experiment. More feasible than the INADEQUATE for real compounds, but much less (up to a factor 100) less sensitive than a HMBC. Looses resolution in direct (f2) dimension. Looks like HMBC with 2 bond correlation only (1,1-ADEQUATE). Combining HMBC with INADEQUATE gives n,1 ADEQUATE.
3-D NMR:	Adding a second or more incremented delays allows to add a third or more dimension to the experiment: INEPT-t_1-INEPT-t_2-TOCSY-acqusition (t_3) gives a 3D HSQC-TOCSY


HMBC
4.4
4.2
4.0
3.8
3.6
3.4
3.2
ppm


ADEQUATE
ppm
110
115
120
125
130
135
140
H1/C19
H5/C21
4.2
4.0
3.8
3.6
3.4
3.2
3.0
ppm
O
C
23
4
OCH3
1
7
N
5
14
10
18
19
21
12 or 13
6
16
8
11
2
15
20
N
22
12 or 13
9
3
17
O