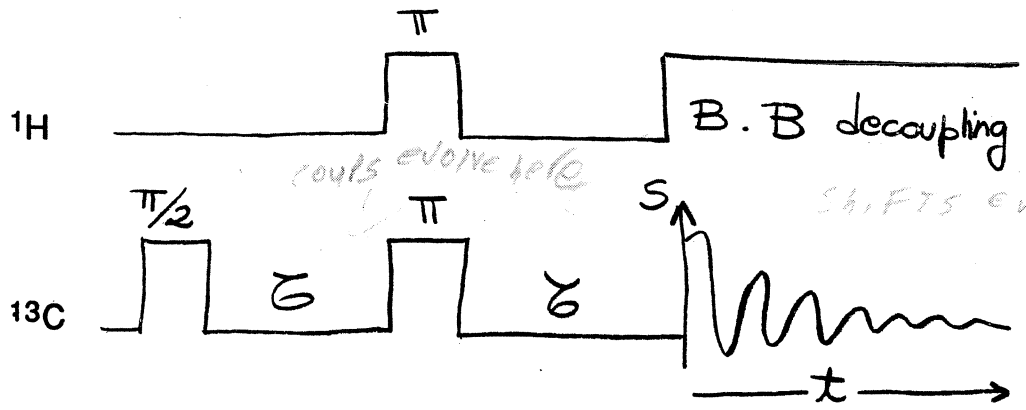
VI: TWO-DIMENSIONAL (2D) NMR

VI.1 INTRODUCTION

Consider again the ^{13}C NMR signal arising from an APT experiment:



The ^{13}C signal detected as a function of time can also be regarded as a quantity depending **parametrically** on τ . The time-domain signal can thus be expressed as

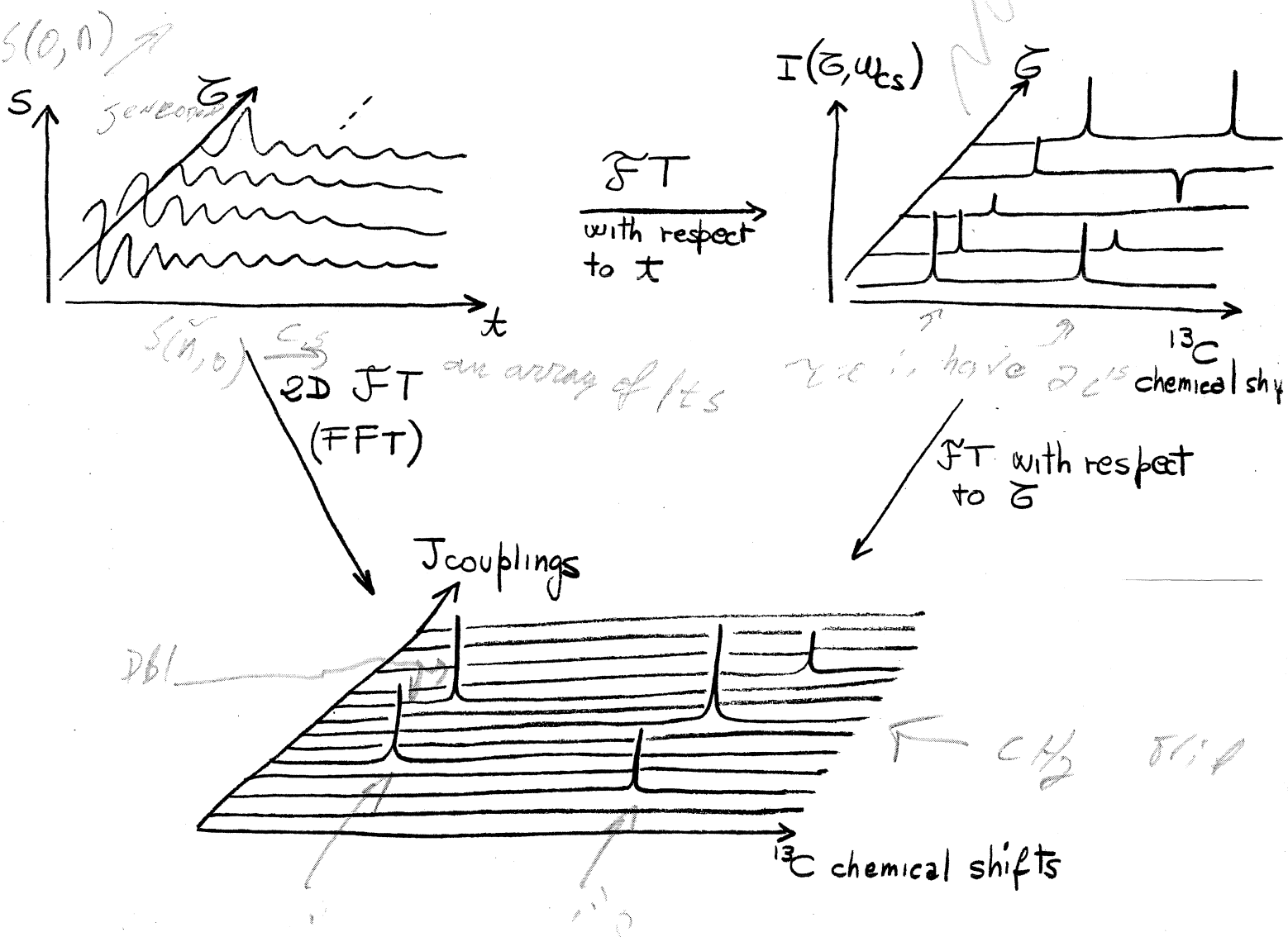
$$S(\tau,t) = \int_{CS}\int_{J} I(\omega_{CS}, J)\cdot e^{i\phi}\cdot dJ\, d\omega_{CS}$$

where ϕ is the classical phase evolved by a ^{13}C coupled to either an α or β proton:

$$\phi = \omega_{CS}\cdot t \pm \frac{J}{2}(2\tau)$$

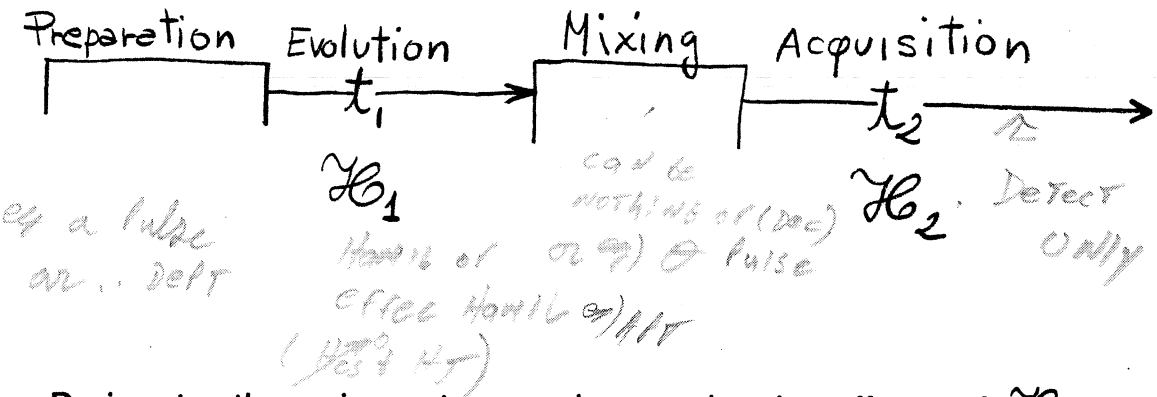
and $I(\omega_{CS},J)$ is the probability of having in our system spins whose chemical shift is ω_{CS} and whose coupling constant is J.

If $S(\tau,t)$ is regarded as a two-dimensional (2D) function depending on 2 independent time variables, then it can be 2D Fourier transformed to provide us with the **2D NMR spectrum** $I(\omega_{cs},J)$



This is the basic type of experiment on which multidimensional NMR is based.

In more general terms, a 2D NMR experiment can be represented by 4 periods:



-During t_1, the spin system evolves under the effects of $\mathcal{H}_1$
-During t_2, the spin system evolves under the effects of $\mathcal{H}_2$
-The preparation period is used to start the evolution with $\mathcal{H}_1$
-The mixing period is used to "turn off" $\mathcal{H}_1$ and "turn on" $\mathcal{H}_2$

If the system can be described in classical terms $\mathcal{H}_1$ and $\mathcal{H}_2$ will have associated precession frequencies of a magnetization vector ν_1 and ν_2, and after 2D FFT we get $I(\nu_1, \nu_2)$

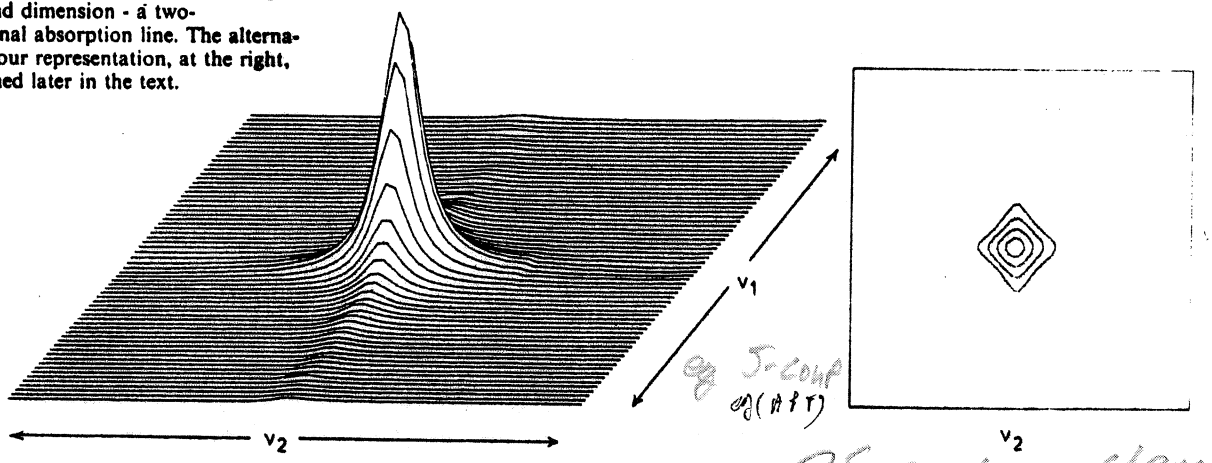


Figure 8.4 The result of transforming in the second dimension - a two-dimensional absorption line. The alternative contour representation, at the right, is explained later in the text.

In contrast with the APT example, the overwhelming majority of solution 2D NMR experiments correlate a **chemical shift evolution in t_1 with a chemical shift evolution in t_2**

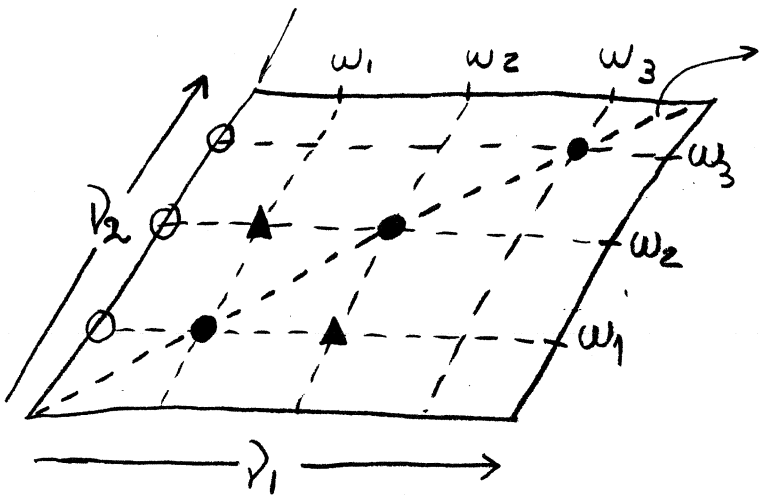
Peaks and line shapes in 2D NMR: Consider a 2D NMR experiment that yields a signal of the type

sum of oscillating fungs

$$S(t_1,t_2)=\int_{t_2>0}\int_{t_1>0} I(\nu_1,\nu_2)\,e^{i\nu_1 t_1}\,e^{i\nu_2 t_2}\,dt_1\,dt_2$$

After 2D exponential weighting and $\mathcal{F}$T, we get in homonuclear correlation experiments:

axial peaks all have same freq. over t_1, don't evolve during T_1 eg) pops of 0,Q,C,



diagonal (only if ν_1 and ν_2 refe to homonuclear chemical shifts)

● : diagonal peaks coh of H' and (removed H

▲ : cross peaks

○ : axial peaks

coh's that started being one kind of Fre (T_1) & changed to Diff during (T_2)

$S(1) \rightarrow S(2)$ } During
& $S(2) \rightarrow S(2)$ }

$I(\omega_2,\omega_2)$: Peaks arising from a coherence that was precessing at a rate ω_2 during t_1 and continued at the same rate during t_2

$I(\omega_1, \omega_2)$: Peaks arising from coherences that were transfered from a precession rate ω_1 to a rate ω_2 during the mixing: **The important part containing the structural or dynamic information.**

$I(0, \omega_2)$: Peaks arising from zero-quantum coherences or populations excited during the mixing

If Quiet Tomorrow exchange Processes, exchange = 0

eg) exch. over time, $1/\tau_1 = 1/\tau_2$ eg) 10% made exch.

∴ correlates to how many made it

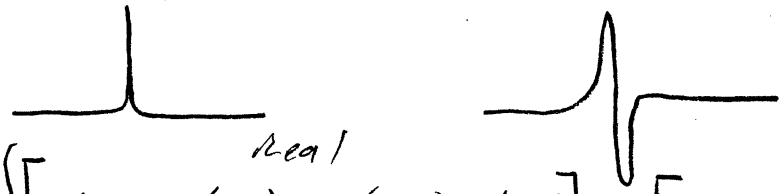
Line Shapes

The line shape of these peaks is different from the one observed in 1D NMR. Indeed, after the Fourier transform we have:

Axis 1 Axis 2

$$I(\omega_i, \omega_j) = S_0 \left[A(\omega_i) + i\,D(\omega_i)\right] \cdot \left[A(\omega_j) + i\,D(\omega_j)\right]$$

with $$A(\omega_i) = \frac{T_2}{(\nu - \omega_i)^2 + T_2^2} \quad ; \quad D(\omega_i) = \frac{\nu - \omega_i}{(\nu - \omega_i)^2 + T_2^2}$$

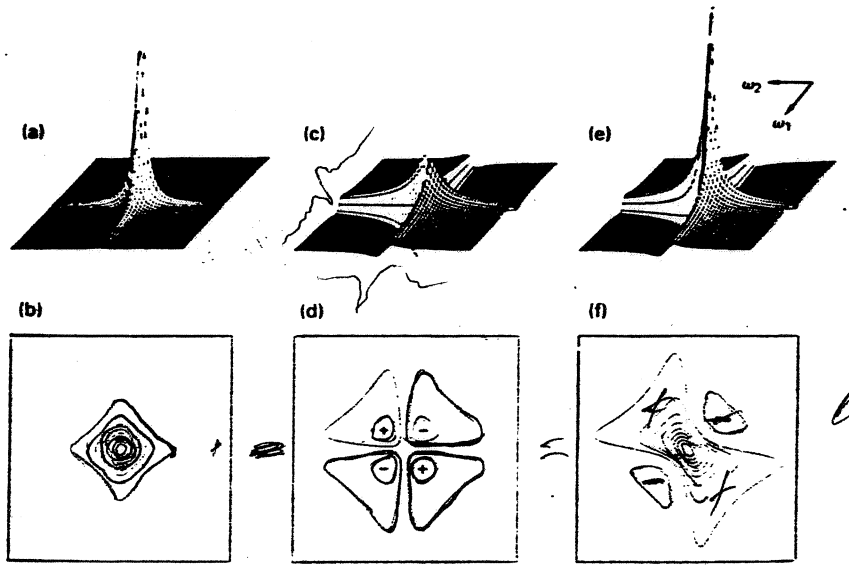


$$\Rightarrow I(\omega_i, \omega_j) = S_0 \left\{ \left[A(\omega_i)A(\omega_j) - D(\omega_i)D(\omega_j)\right] + i\left[A(\omega_i)D(\omega_j) - A(\omega_j)D(\omega_i\right.\right.$$

$A(\omega_i)A(\omega_j)$ − $D(\omega_i)D(\omega_j)$ = $A_iA_j - D_iD_j$

purely absorptive peak — purely dispersive peak — mixed phase peak

Make one of Disp's = 0 → Purely Abs



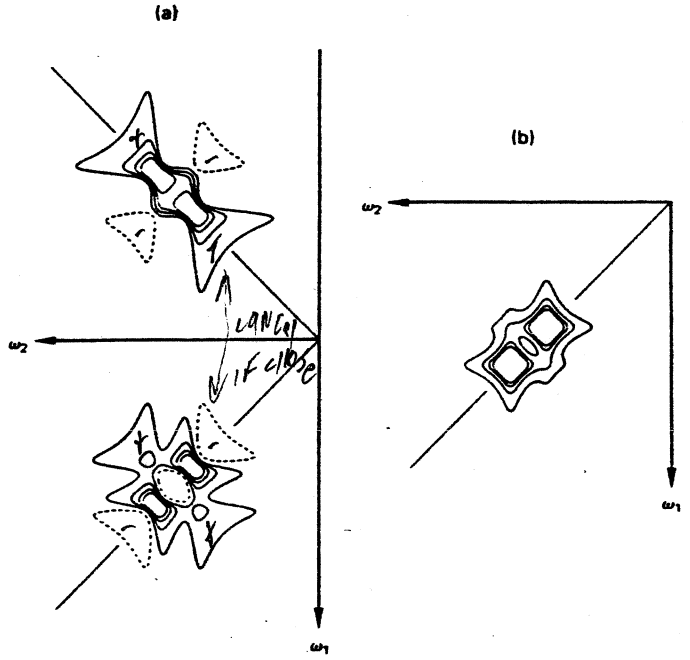
Got this

FIG. 6.5.1. Peakshapes in 2D spectra: (a) and (b) pure 2D absorption $a_{ni}(\omega_1)a_{ni}(\omega_2)$; (c) and (d) pure negative 2D dispersion $-d_{ni}(\omega_1)d_{ni}(\omega_2)$; (e) and (f) mixed phase peakshape, also known as 'phase-twisted' peakshape, consisting of a superposition $a_{ni}(\omega_1)a_{ni}(\omega_2) - d_{ni}(\omega_1)d_{ni}(\omega_2)$.

1D A + D

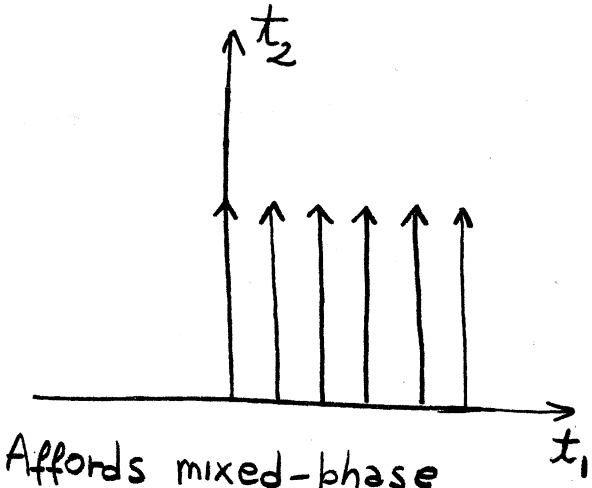
Could Phase This But Now These are Mixed.

Overlap of mixed-phase line shapes produces signal cancellation:



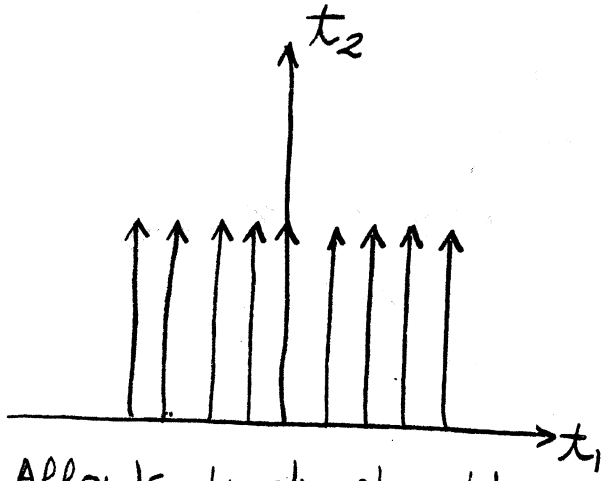
6.5.2. (a) Superposition of a pair of lines with mixed phases: in the lower quadrant, the resonance positions are displaced along the positive diagonal ($\Delta\Omega_1 = \Delta\Omega_2 = 4\lambda_1 = 4\lambda_2$, i.e. displaced by twice the full line-width at half-height), leading to destructive interference due to the overlap of negative dispersion lobes with positive absorption components. In the upper quadrant, the resonance positions are displaced along the negative diagonal ($\Delta\Omega_1 = -\Delta\Omega_2 = 4\lambda_1 = 4\lambda_2$), leading to constructive interference. (b) Superposition of two pure 2D absorption lines, displaced by $\Delta\Omega_1 = \Delta\Omega_2 = 4\lambda_1 = 4\lambda_2$. The contour levels shown correspond to 22, 16, 10, 4, and −4 per cent of the maximum height of an isolated peak. Negative contours are drawn with dashed lines.

To get purely absorptive line shapes we have to make the dispersive component along either ν_1 or ν_2 equally zero. This is equivalent to ask for a time-domain sampling involving a t_1- or t_2-echo.



Affords mixed-phase line shapes

$D(\omega_2), D(\omega_1) \neq 0$



Affords purely-absorptive line shapes

$D(\omega_2) \neq 0;\ D(\omega_1) = 0$

We will come back to this topic later.

Need this to do F1e of Quadrature?

Recall

VI.2 2D CORRELATIONS VIA J-COUPLINGS

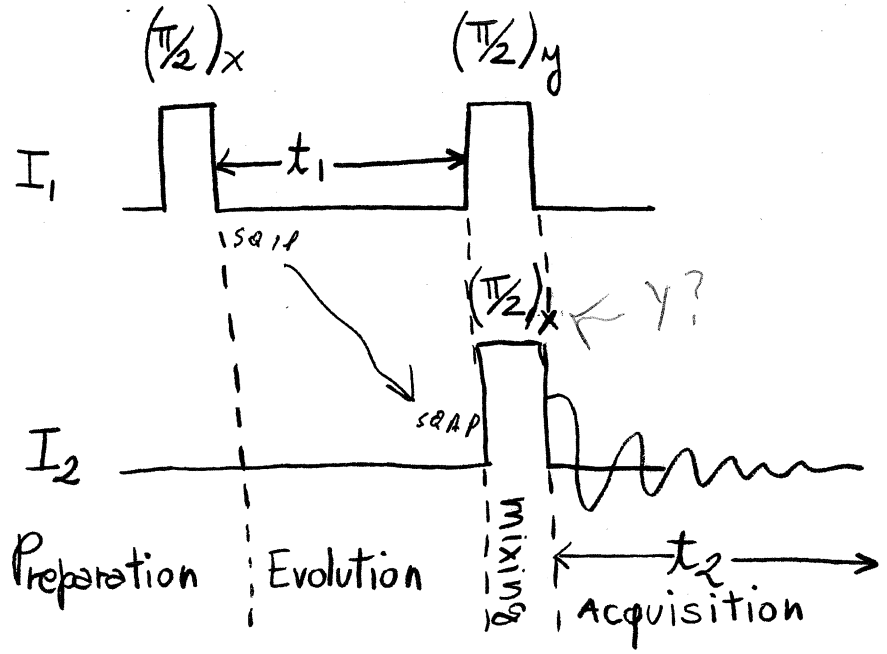
When trying to correlate the chemical shifts of 2 sites, there are 3 mechanisms on which the transfer of coherence between t_1 and t_2 can rely:

i) **J-couplings**; which originate the family of **COSY** (**CO**rrelated **S**pectroscop**Y**)-type experiments, showing cross-peaks between pairs of coupled sites.

ii) **Dipole-Dipole Relaxation**; which originates the family of **NOESY** (**NOE S**pectroscop**Y**)-type experiments, showing cross-peaks between spatialy-proximate sites. which H's → ENZ, BINDING INFO → H/D INFO

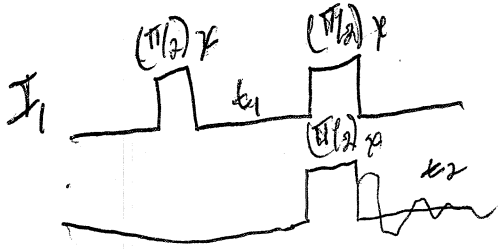
iii) **Chemical Exchange**; which originates 2D exchange NMR spectra, very similar to NOESY spectra except by the fact that cross-peaks arise due to chemical exchange processes. → 2D NMR ← # exch. matrix

We will start by focusing on COSY-type experiments. Cross peaks in COSY arise from the excitation of multiple-spin antiphase coherence terms like those that we saw in the basic heteronuclear coherence transfer sequence:



INEPT → KEY TO 2D NMR $^1H_A \rightarrow {}^1H_B \rightarrow {}^{13}C_B$ . . .
IF NO π PULSE, ∴ STILL ENCODE CHEM SHIFT

Let's analyze the evolution of ρ , assuming chemical shifts {ω1, ω2} for {I1, I2}, and a first-order coupling J



eg) $S_1 = {}^1H$

$S_2 = {}^{13}C$

I_{z_1}

$\downarrow (\pi/2)_x$

I_{y_1} evolves

chemical shift; t_1

$-I_{x_1}\sin(\omega_1 t_1)$ — coupling; t_1

$-I_{x_1}\sin(\omega_1 t_1)\cos\left(\frac{Jt_1}{2}\right)$ $\downarrow (\pi/2)_y$ $-I_{z_1}\sin(\omega_1 t_1)\cos\left(\frac{Jt_1}{2}\right)$ → population

$-2I_{y_1}I_{z_2}\sin(\omega_1 t_1)\sin\left(\frac{Jt_1}{2}\right)$ $\downarrow (\pi/2)_y$ $2I_{y_1}I_{x_2}\cos(\omega_1 t_1)\sin\left(\frac{Jt_1}{2}\right)$ → single + double-quantum coherence

population + single + double-quantum coherence → not observable

$I_{y_1}\cos(\omega_1 t_1)$ — coupling; t_1

$-2I_{x_1}I_{z_2}\cos(\omega_1 t_1)\sin\left(\frac{Jt_1}{2}\right)$ $\downarrow (\pi/2)_y$ $-2I_{z_1}I_{y_2}\cos(\omega_1 t_1)\sin\left(\frac{Jt_1}{2}\right)$

a.p. 1 vs 2

a.p. 2 vs. 1

$I_{y_1}\cos(\omega_1 t_1)\cos\left(\frac{Jt_1}{2}\right)$ $\downarrow (\pi/2)_y$ $I_{y_1}\cos(\omega_1 t_1)\cos\left(\frac{Jt_1}{2}\right)$

precesses at ω_1 during t_2; we ignore it for this particular example

coupling; t_2

$-2I_{z_1}I_{y_2}\cos(\omega_1 t_1)\sin\left(\frac{Jt_1}{2}\right)\ldots$ antiphase coherence not observable

$-2I_{x_2}\cos(\omega_1 t_1)\sin\left(\frac{Jt_1}{2}\right)\sin\left(\frac{Jt_2}{2}\right)$

chem. shift; t_2

$2I_{y_2}\cos(\omega_1 t_1)\sin(\omega_2 t_2)\sin\left(\frac{Jt_1}{2}\right)\sin\left(\frac{Jt_2}{2}\right)$

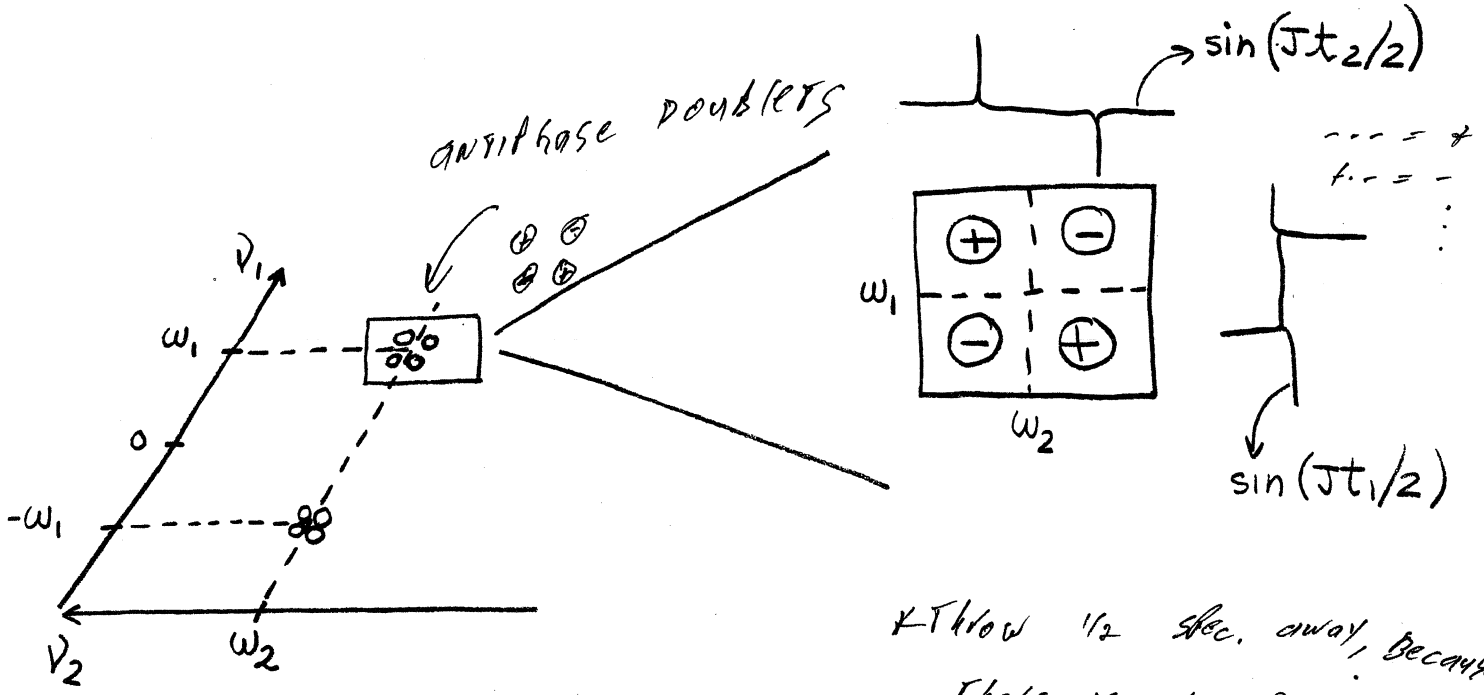
$-2I_{x_2}\cos(\omega_1 t_1)\cos(\omega_2 t_2)\sin\left(\frac{Jt_1}{2}\right)\sin\left(\frac{Jt_2}{2}\right)$

If we only detect the signal from spin 2 at a frequency ω_2 =>

$$S(t_2) \propto Tr\left(\rho \cdot I_{2+}\right) = \cos(\omega_1 t_1)\sin(Jt_1/2)\cdot\sin(Jt_2/2)\cdot e^{i\omega_2 t_2}$$

Note that the frequency ω_1 can be measured by stepping t_1 even though sampling takes place at a different Larmor frequency!

The line shape of these cross-peaks:



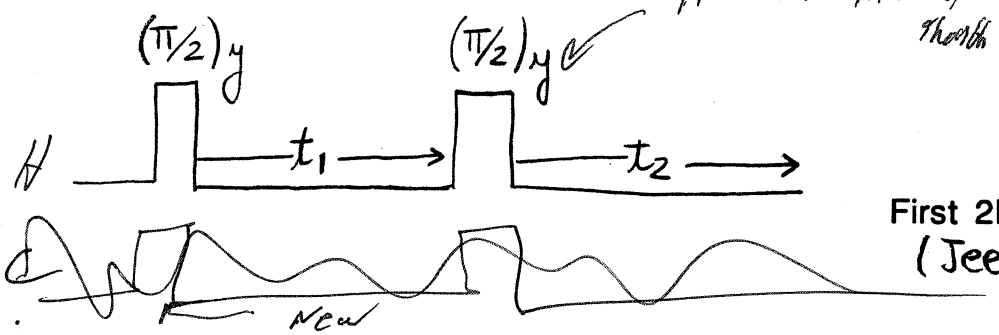
* Throw 1/2 spec. away, because there is no Quad Detect along T_1 (can't distinguish phase).
∴ No Q. Det, ∴ use large offsets remember

At along T_1, Detect cos in both the + & − T_1's 90° get ampl't. modulation Detect
Not Phase Detect,

Can treat 'H systems as Heteronuc case at very high fields eg)

VI.3 THE 2D HOMONUCLEAR COSY NMR EXPERIMENT

Let's consider now a **homonuclear** system; i.e., a system where the first ($\pi/2$) pulse excites both spins and where in the acquisition period we detect the signal from both spins. The simplest sequence for retrieving a COSY NMR spectrum from such a system



The analysis of ρ in this system is very similar to the one described above except for 2 differences:

i) In the previous example we had that $\rho_0 = I_{z_1}$; now $\rho_0 = I_{z_1} + I_{z_2}$ => there is a 2nd signal to consider.

ii) Since now both pulses have the same rf phase (previously we had that $(\pi/2)_x$ - $(\pi/2)_y$), in the present sequence we have to consider terms proportional to $\sin(\omega_1 \cdot t_1)$ (instead of the cosines that we kept before).

Overall, the terms observable during t_2:

Originating in I_{z_1} — Originating in I_{z_2}

$I_{x_1}\ S^1_{\omega_1}\ C^1_J\ C^2_J\ S^2_{\omega_1}$ ← diagonal peaks → $I_{x_2}\ S^1_{\omega_2}\ C^1_J\ C^2_J\ S^2_{\omega_2}$

$I_{y_1}\ S^1_{\omega_1}\ C^1_J\ C^2_J\ C^2_{\omega_1}$ ← diagonal peaks → $I_{y_2}\ S^1_{\omega_2}\ C^1_J\ C^2_J\ C^2_{\omega_2}$

$I_{x_2}\ S^1_{\omega_1}\ S^1_J\ S^2_J\ S^2_{\omega_2}$ ← cross peaks → $I_{x_1}\ S^2_{\omega_2}\ S^1_J\ S^2_J\ S^2_{\omega_1}$

$I_{y_2}\ S^1_{\omega_1}\ S^1_J\ S^2_J\ C^2_{\omega_2}$ ← cross peaks → $I_{y_1}\ S^1_{\omega_2}\ S^1_J\ S^2_J\ C^2_{\omega_1}$

C_J; $S^1_J = \cos(Jt_1/2)$; $\sin(Jt_1/2)$ $\quad C^2_J$; $S^2_J = \cos\left(\frac{Jt_2}{2}\right)$; $\sin\left(\frac{Jt_2}{2}\right)$

$S^1_{\omega_1}$; $S^1_{\omega_2} = \sin(\omega_1 t_1)$; $\sin(\omega_2 t_2)$ $\quad S^2_{\omega_i}$; $C^2_{\omega_i} = \sin(\omega_i t_2)$; $\cos(\omega_i t_2)$

Probs 1) no Quad. detect
2) have axial peaks
x-mag which relaxed during T_1
∴ 90° → Takes back to x-y w/ zero

Two important points to notice:

_Since t_1 modulation appears as sines or cosines, non-quadrature detection along ω_1 takes place => one has to work off-resonance and throw away half the points. To avoid working off-resonance one has to phase cycle the relative phases of the ($\pi/2$) pulses (see below)

_Whereas the t_1 modulation of the diagonal peaks comes as products of a cosine and a sine factor ($\sin(\omega_i \cdot t_1) \cdot \cos(J \cdot t_1/2)$), the cross-peaks are doubly sine modulated ($\sin(\omega_i \cdot t_1) \cdot \sin(J \cdot t_1/2)$).

Intersection

If we recall some trigonometric relationships, it is possible to show that the t1 modulation of the

2 Transfer funcs

* Can never have 2D spectrum purely ABS.

diagonal-peaks

cross-peaks

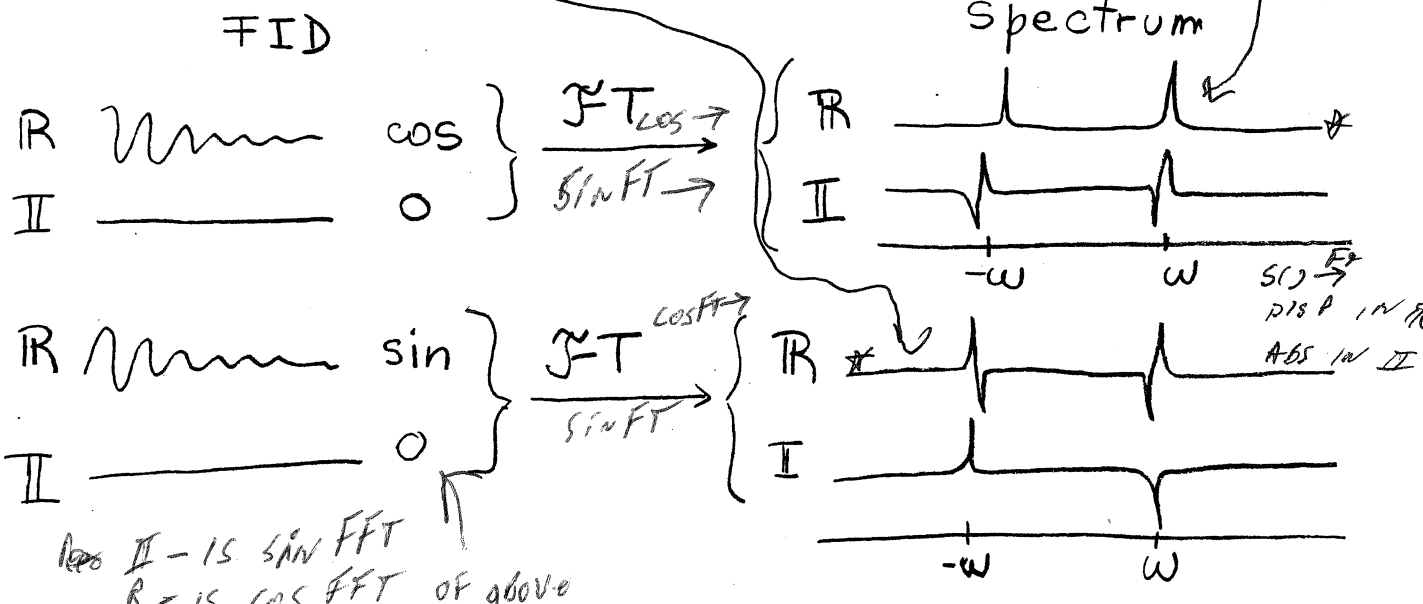
$$\sin(\omega_1 t_1)\cos\left(\frac{J t_1}{2}\right) = \quad e^{i} + e^{-i} \} \text{ Non-quad det.} \qquad \sin(\omega_1 t_1)\sin\left(\frac{J t_1}{2}\right) =$$

$$= \frac{1}{2}\left\{\sin\left[\left(\omega_1 - \frac{J}{2}\right)t_1\right] + \sin\left[\left(\omega_1 + \frac{J}{2}\right)t_1\right]\right\} \qquad = \frac{1}{2}\left\{\cos\left[\left(\omega_1 - \frac{J}{2}\right)t_1\right] - \cos\left[\left(\omega_1 + \frac{J}{2}\right)t_1\right]\right.$$

Non-quad detect → 4 peaks

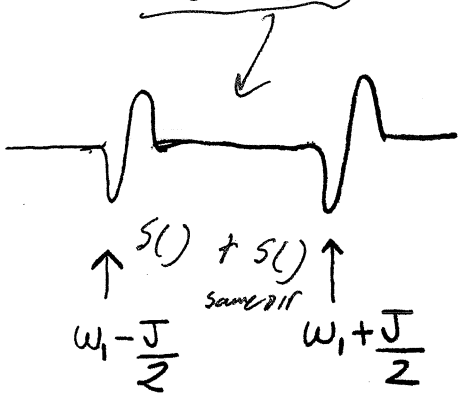
1st Throw half signal away Then add

Using what we saw in Section II about $\mathcal{F}$T:

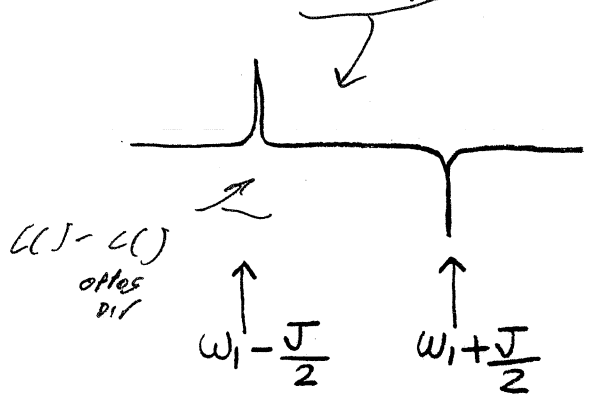


It can be shown that the line shapes of the COSY peaks for positive ω_1 are

at the diagonal-peaks

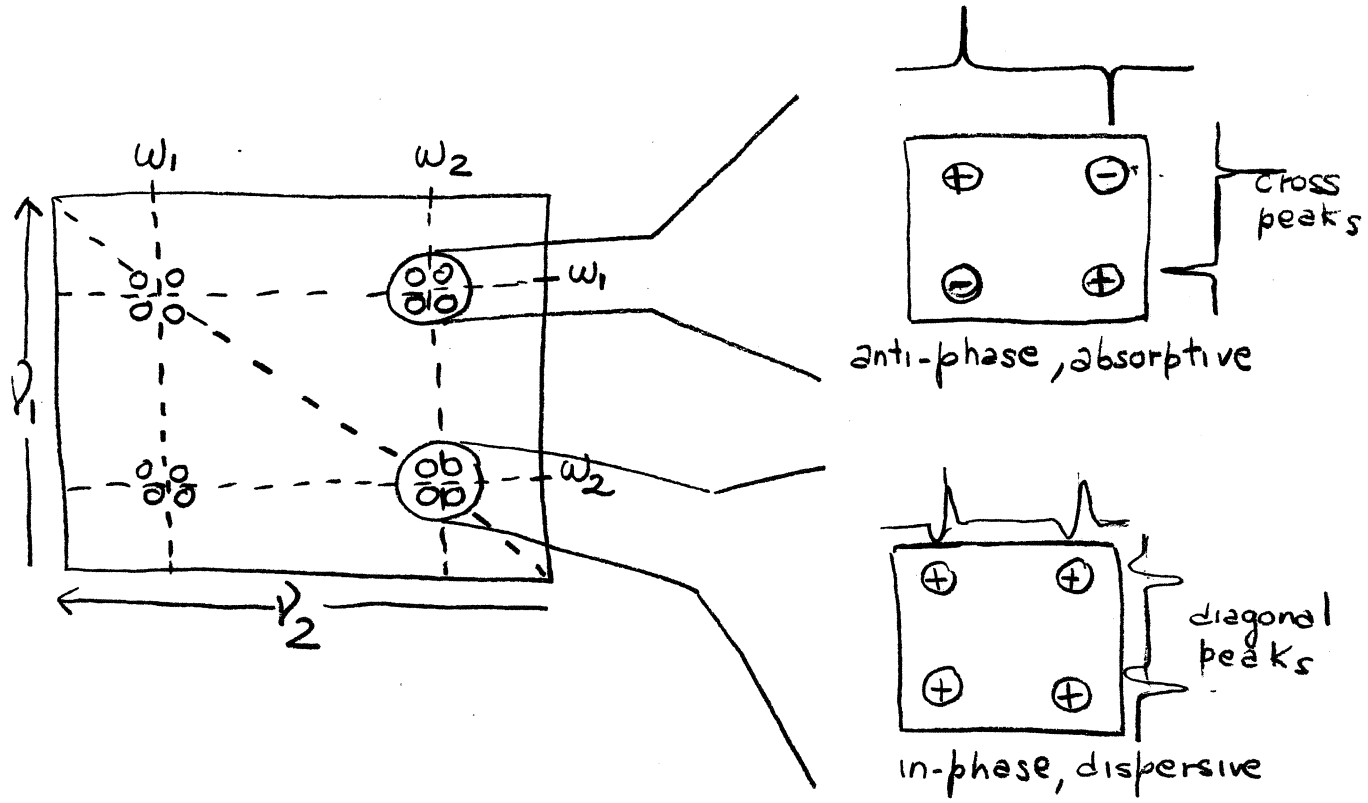


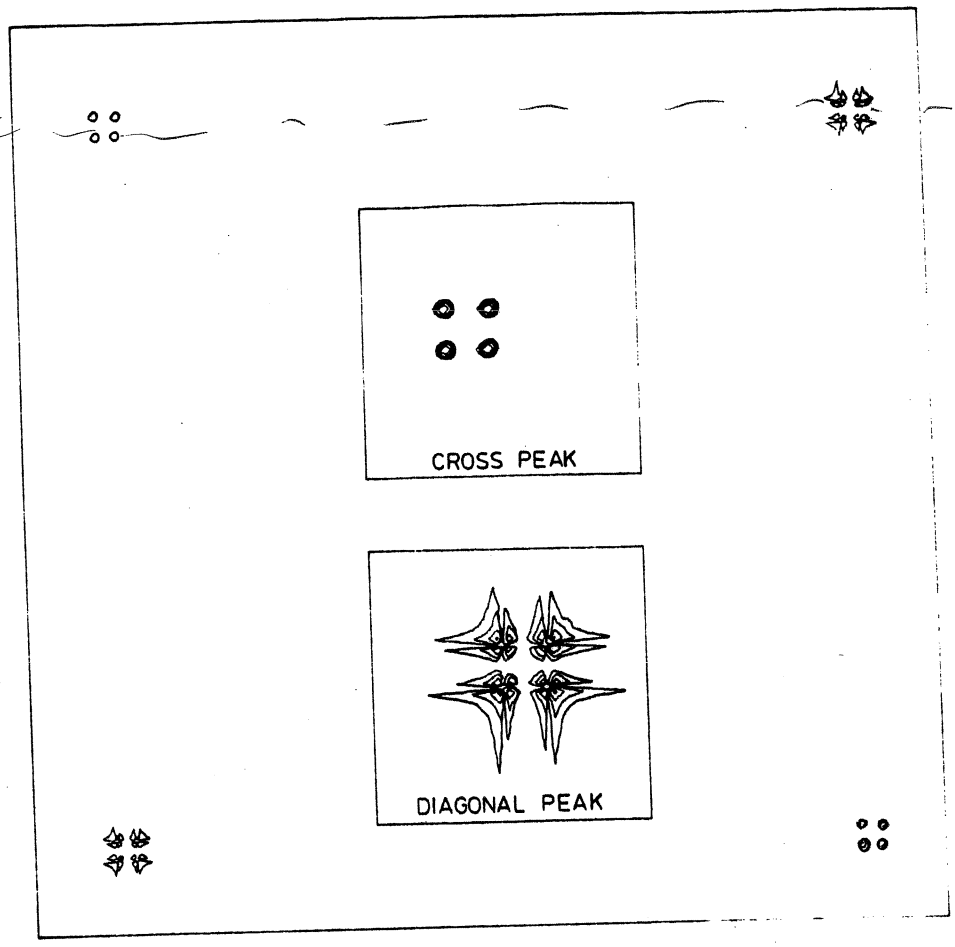
at the cross-peaks



Thus, it follows that **it is not possible to record a completely phased COSY spectrum**. We can have along ν_1 either an absorptive diagonal and dispersive cross-peaks or vice-versa. Since the most important information lies in the cross-peaks, the latter choice is usually implemented.

The **transfer functions** multiplying each operator allow us to reconstruct the multiplet line shapes of the total 2D spectrum.

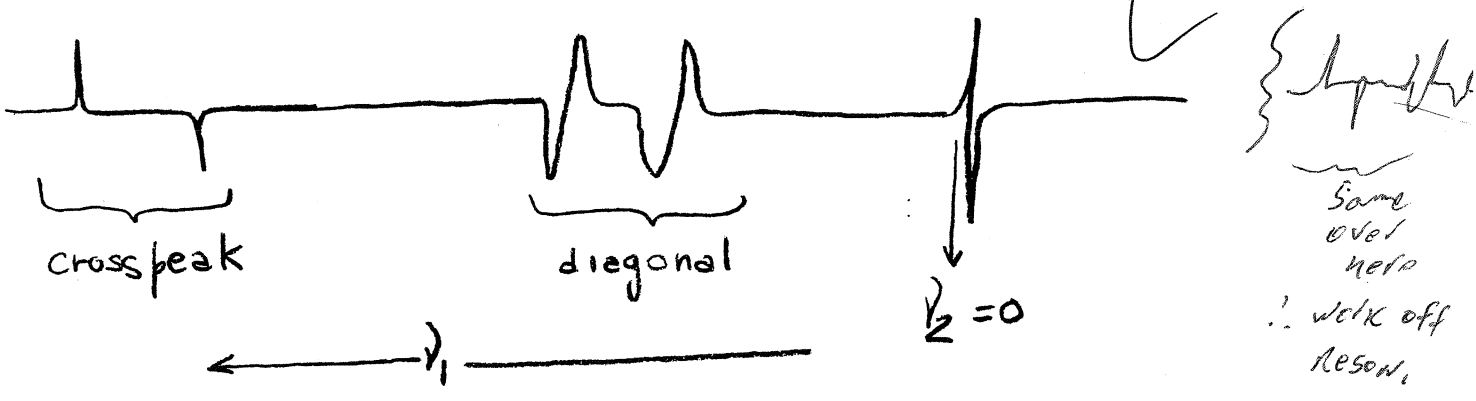




cross section slice

Figure 8.25 Phase-sensitive COSY spectrum of an AX system; positive contours are black and negative red. Note how the cross-peaks have been adjusted into pure absorption phase, with multiplet components in antiphase (inset in contour plot). The diagonal peaks are dispersive, and overlap of the wide dispersion lines leads to characteristic 'angel' shapes.

A cross-section at $\nu_2 = \omega_1$



Plot — Diags IF To strong overlap w/ C.P's ∴ hide them

This axial peak arises from magnetization that relaxed during t_1 or was not excited by the first pulse

AXIAL-peaks → artifacts eg) imperfect 90°s, . . .

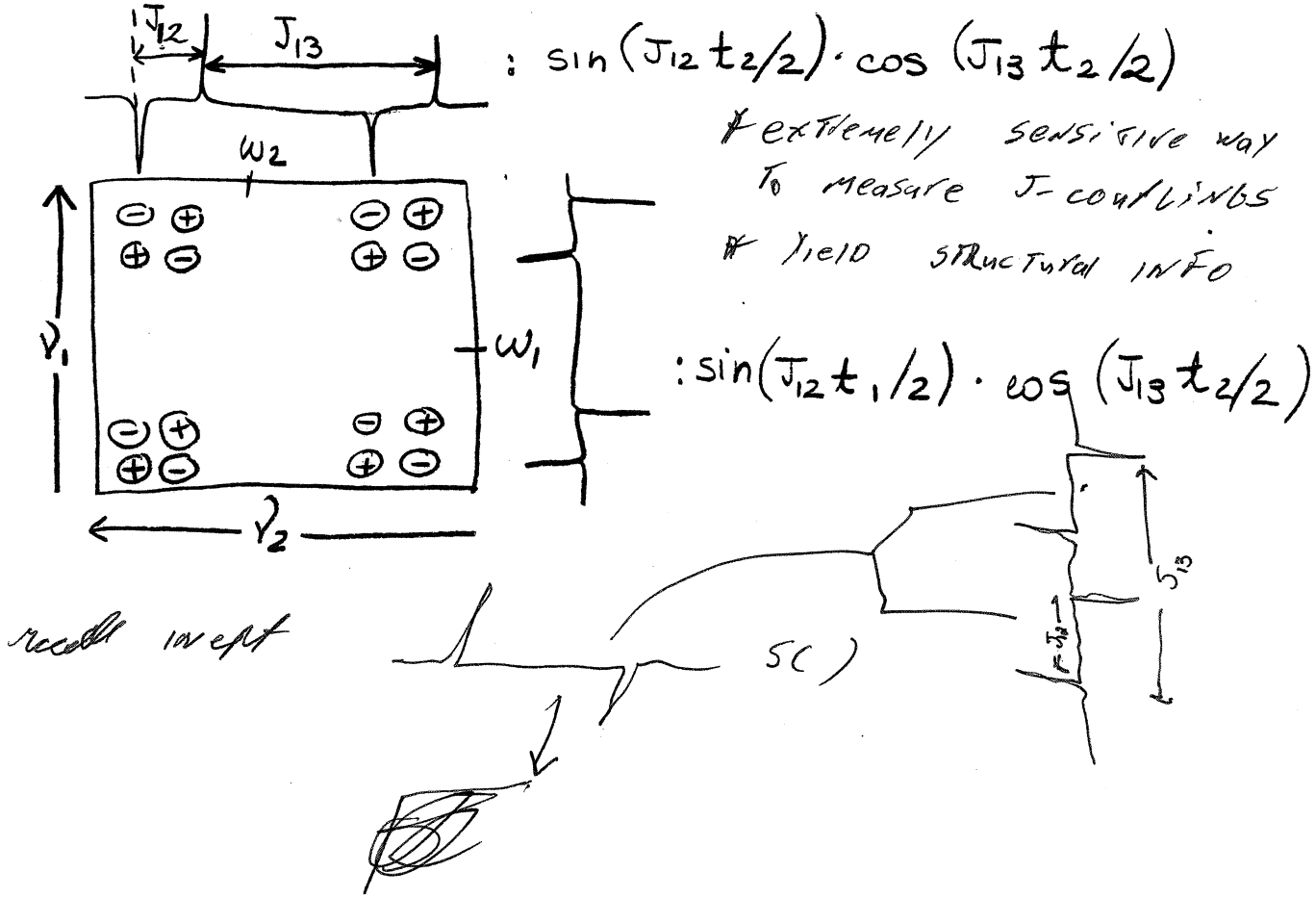
We investigate now how does an (ω_1,ω_2) cross peak look like in the presence of a coupling to a 3rd spin I_3. An (ω_1,ω_2) cross peak comes from the transfer

$$I_{z_1} \xrightarrow{(\pi/2)_x} I_{y_1} \xrightarrow{J I_{z_1} I_{z_2} t} 2I_{x_1} I_{z_2} \xrightarrow{(\pi/2)_x} 2I_{z_1} I_{y_2} \longrightarrow I_{x_2}$$

Going back to Section V, recall that the evolution of bilinear terms in the presence of coupling to a 3rd spin changes to

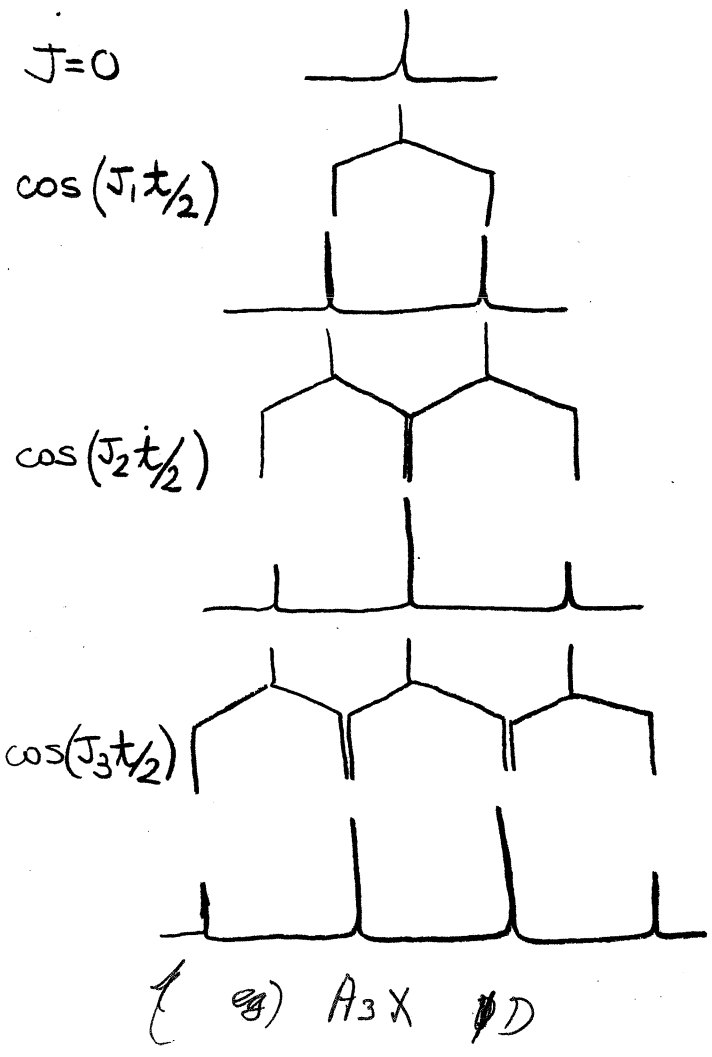
$$2I_{x_1} I_{z_2} \xrightarrow{J I_{z_2} I_{z_3} t} 2I_{x_1} I_{z_2} \xrightarrow{J I_{z_1} I_{z_3} t} 2I_{x_1} I_{z_2} \cos(J_{13} t/2) + 4I_{y_1} I_{z_2} I_{z_3} \sin(J_{13} t/2)$$

One therefore has to differentiate between the **active** coupling J_{12} originating the cross peak, and the **passive** coupling J_{13} that splits this cross peak. **Each passive coupling J contributes a cos(Jt/2) term to the transfer function.** The total line shape of the final COSY cross peak then becomes



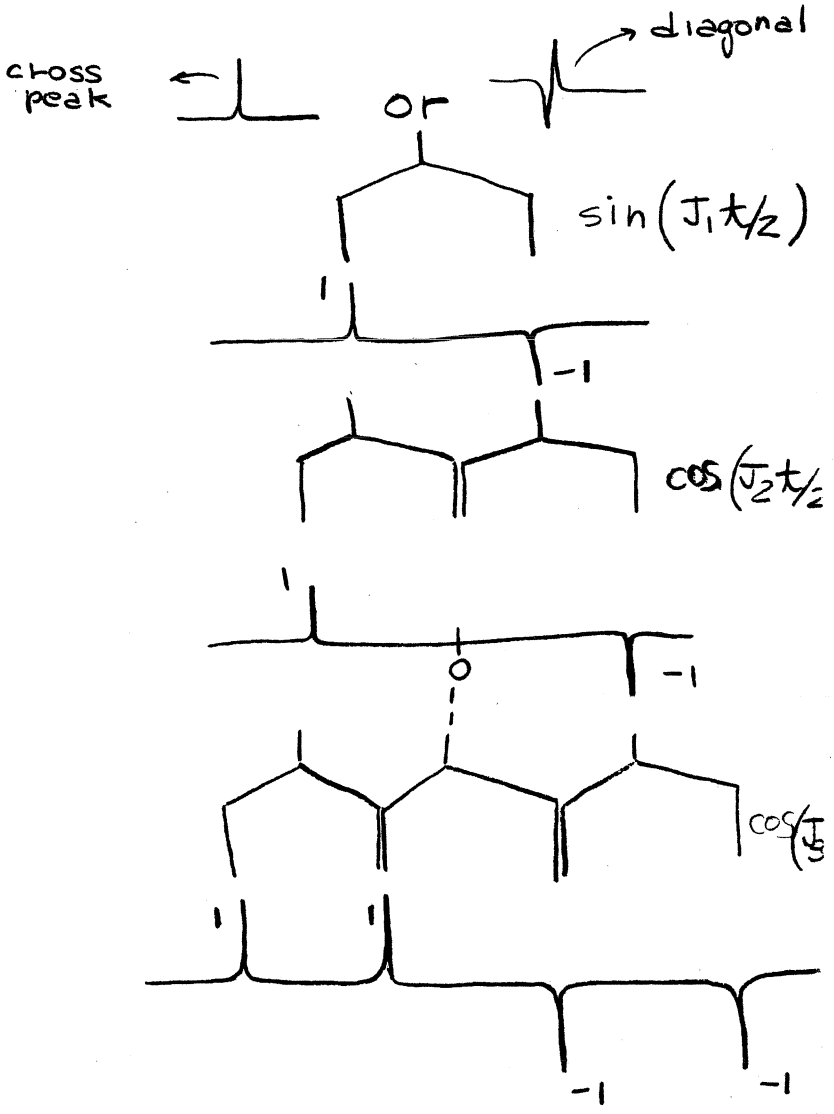
If we have a cross peak arising from an A_3X system, the signal at ω_X:

In normal 1D NMR



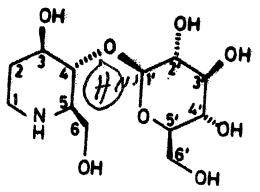
(eg) A_3X 1D

In 2D COSY NMR



VI.4 2D COSY NMR: AN EXAMPLE

In spite of the complexity of its spectra, the 2-pulse H,H-COSY experiment has been and still is one of the most important tools for structural characterization in chemistry. We illustrate its use by ellucidating the spectrum of the natural product:



1

Its 1D ^1H NMR spectrum is very complex

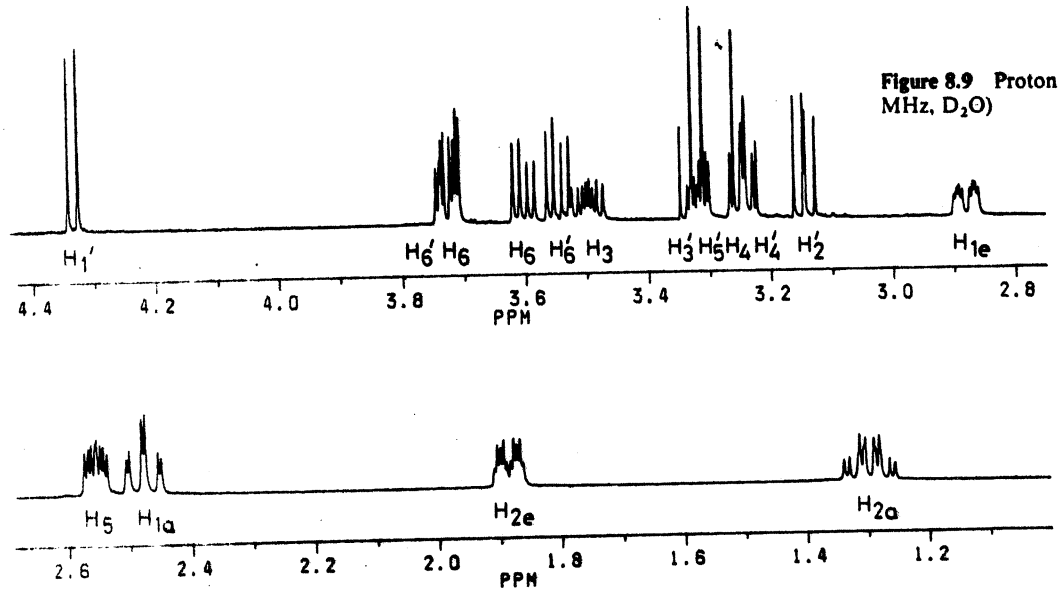
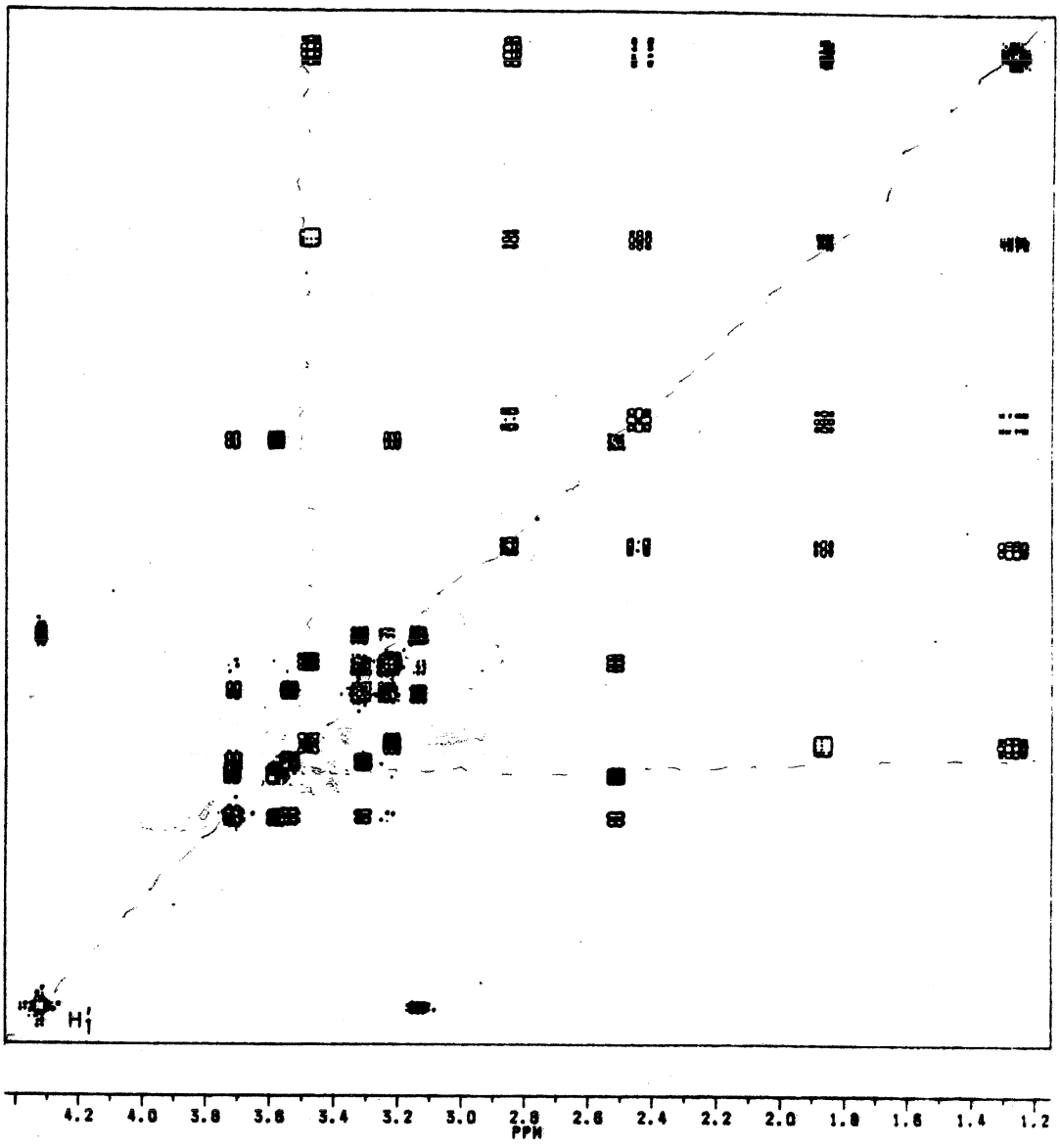
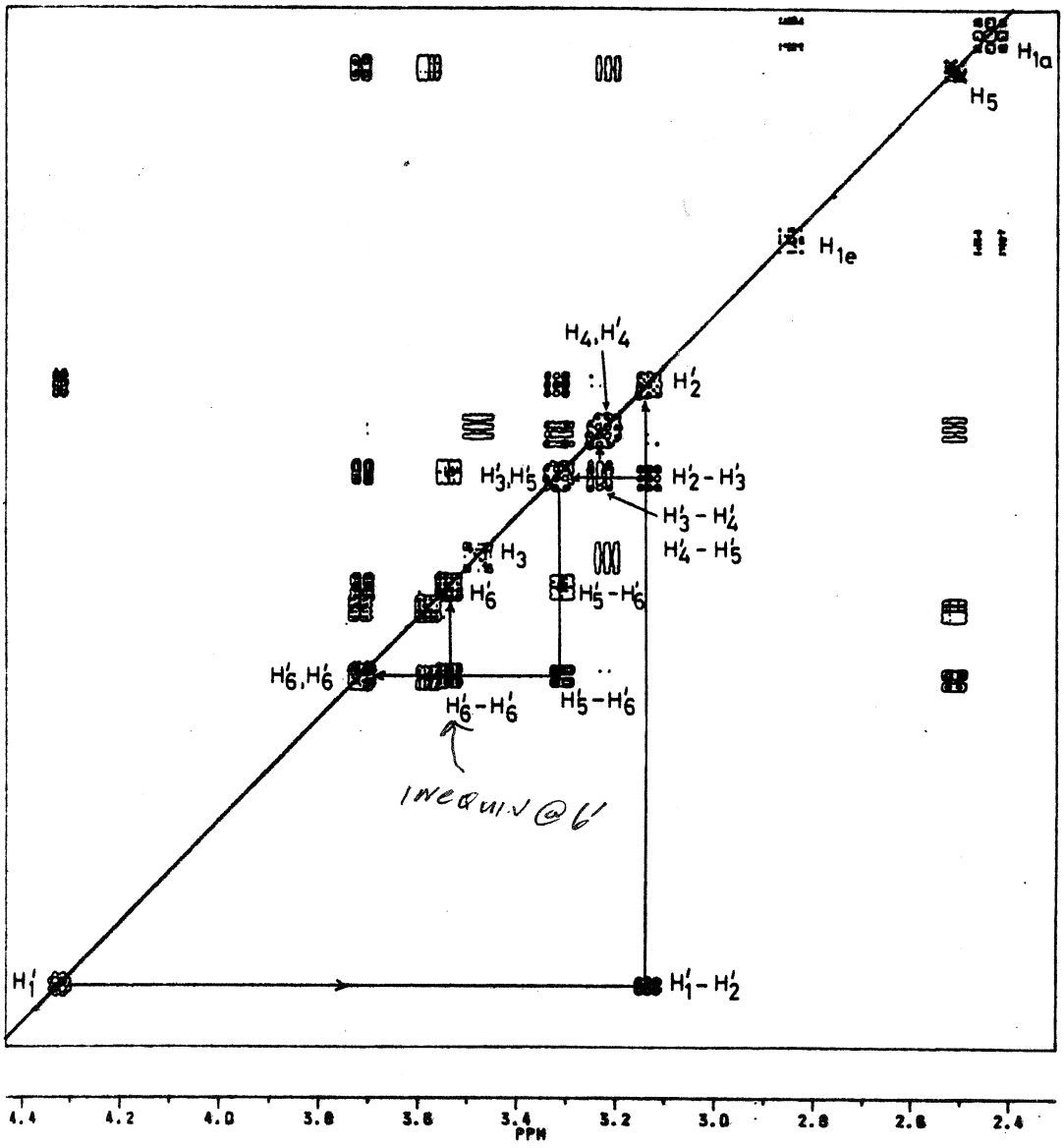


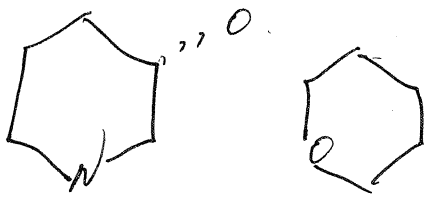
Figure 8.9 Proton spectrum of 1 (500 MHz, D_2O)

The complete 2D H,H-COSY spectrum of the compound



Once the origin of one peak is known, the remaining protons in the molecule can then be traced back by following the molecular connectivities indicated by the cross-peaks. For the sugar region (higher ppm's) for instance





VI.5 THE SPHERICAL BASIS SET; COHERENCE ORDERS

As we have seen it so far, the COSY experiment has a nunber of drawbacks:

i) Non-quadrature detection in t_1 (I_x modulation rather than I_+ or I_-)
ii) Line shapes cannot be made purely absorptive (dispersive diagonals)
iii) Axial peak artifacts along v_1 (not originating in DC offsets)

} want to elim. all these

Eliminate By phase cycling.

We will try to solve these problems by shifting the phases of the various rf pulses, but before we do that we have to investigate how the different components of the density matrix (spin coherences) behave under the effects of phase shifts.

We saw in Section V.1 that under the effects of the chemical shift, the evolution of a cartesian I_x operator is given by:

$$I_x \xrightarrow{\Delta\omega I_z t} I_x \cos(\Delta\omega t) + I_y \sin(\Delta\omega t) = \frac{I_+}{2} e^{-i\Delta\omega t} + \frac{I_-}{2} e^{+i\Delta\omega t}$$

phase-cycled out ↑ detected ↑

$\{I_x, I_y, I_z, \mathbb{1}\}$ constituted a basis set for ρ: **cartesian basis of a spin 1/2**

$\{I_+, I_0, I_-, \mathbb{1}\}$, with $I_0 = I_z$, also constitutes a basis set for ρ: it is called the **spherical basis set**

Switch your bases

can describe any spin 1/2 with this

Similarly, we can write a spherical basis set for a 2 spin system I-S:

$$\mathbb{1}, I_0, S_0, I_0S_0, I_+S_-, I_-S_+, \underbrace{S_+, I_+, S_-, I_-, I_+S_0, I_0S_+, I_-S_0, I_0S_-}_{\text{single-quantum coherences}},$$

S.Q. w/ Hasc

S.Q. H.F.

populations

spin-order

zero-quantum coherences

$$\underbrace{I_+S_+, I_-S_-}_{\text{double-quantum coherences}}$$

10) With the following parameters

① $J\tau' = 1/8$ ② $J\tau' = 1/4$ ③ $J\tau' = 3/8$

these INEPT experiments can be used to get the -$\overset{1}{C}$H-, -CH_2- and -CH_3 subspectra of a compound

$-\overset{1}{C}H \longrightarrow ②$

$-CH_2- \longrightarrow ① - ③$

$-CH_3 \longrightarrow ① + ③ - \sqrt{2} \cdot ②$

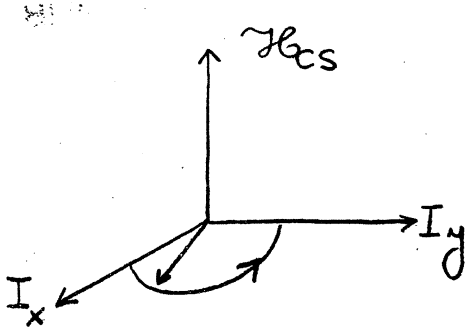
Calculate the intensities of all these subspectra if the J couplings are 10% smaller than their ideal values (i.e., J = 126 Hz instead of 140 Hz).

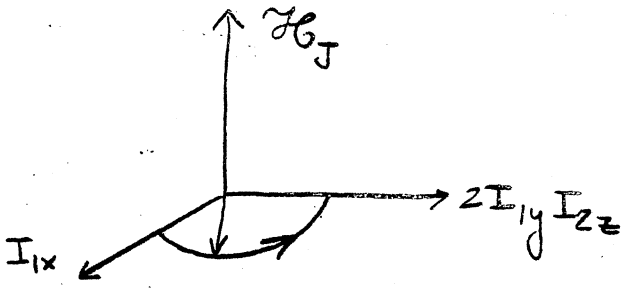
11) ^{13}C spectral editing can be carried out in DEPT by doing three experiments with $\theta_1 = \pi/4$, $\theta_2 = \pi/2$, $\theta_3 = 3\pi/4$

i) Calculate the linear combinations that will originate the different spectra.

ii) Analyze the "cross-talk" among the subspectra that occurs if the assumed $\bar{J}$ = 140 Hz and the actual $\bar{J}$ = 120 Hz

The main advantage of the cartesian basis is that it allows us to characterize the evolution of ρ under the effects of Hamiltonians as **rotations** in different spaces





Have problem w/ Kf 01

In some cases however, the spherical basis is more convenient than the cartesian. For instance, it is easy to see that there is no "scrambling " of operators during chemical shift evolution

$$I_\pm \xrightarrow{\Delta\omega I_z t} I_\pm e^{\mp i\Delta\omega t}$$: No mixing of operators

$$I_\pm \xrightarrow{J I_z S_z t} I_\pm \cos\left(\frac{Jt}{2}\right) \pm 2 I_\pm S_z \sin\left(\frac{Jt}{2}\right)$$? see HW

Particularly cumbersome to analyze in the cartesian basis are the consequences of phase-cycling; i.e., of systematically changing the phase of an rf pulse. For instance, I_z gives I_x if the pulse is along the y axis but a **different operator** if the pulse is shifted 90 degrees.

By contrast, the effects of phase shifts on ρ are particularly simple to analyze in the spherical basis set. Consider for instance a pulse sequence that acts on a two-spin system, and which starting from $\rho_0 = I_z$ ends up making an arbitrary state ρ_t:

$$\rho_0 \xrightarrow{U(t)} \rho_t$$

$U(t)$ involves delay, chemical shifts, etc., J-coupl

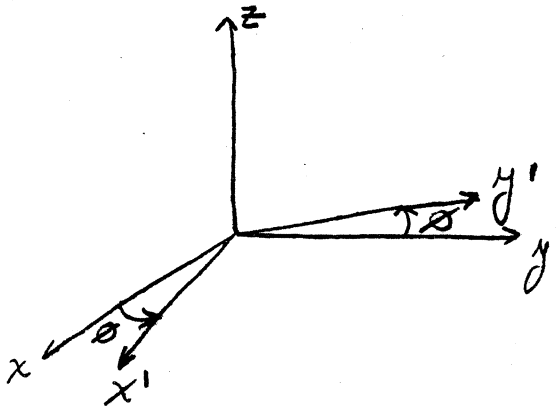
$$\rho_t = a \cdot \mathbb{1} + b \cdot I_0 + c \cdot S_0 + d\, I_0 S_0 + \ldots$$

From Basis set

We want to find out how ρ_t changes if one shifts the phase of **all** the pulses by ϕ degrees. It is possible to find out the effects of this phase shift by analyzing the behavior of each element in the basis set:

$$I_0, S_0, I_0S_0, I_+S_-, \text{ etc.}$$

Notice that a phase shift ϕ in the phase of the rf is equivalent to redefining the rotating frame according to:



From a quantum-mechanical point of view, this rotation by ϕ degrees around the z axis is represented by a rotation operator

$$R = e^{-iF_z\phi} \quad ; \quad F_z = I_z + S_z$$

Total z-ang Mom

The behavior of the different coherence orders under the effects of this operator

populations: $e^{-iF_z\phi}\{I_z, S_z, I_zS_z\}\, e^{iF_z\phi} = \{I_z, S_z, I_zS_z\}$: no change

spin-order

zero-quantum: $e^{-iF_z\phi}\, I_+S_-\, e^{iF_z\phi} = \underbrace{e^{-iI_z\phi}\, I_+\, e^{iI_z\phi}}\ \underbrace{e^{iS_z\phi}\, S_-\, e^{-iS_z\phi}}$

$= I_+ e^{-i\phi} \cdot S_- e^{i\phi} = I_+S_-$: no change

(Def of O.Q.C. No change w/ Phase)

$-1: e^{-iF_z\phi}\{I_-, S_-, I_-S_z, I_zS_-\}e^{iF_z\phi} = e^{i\phi}\{I_-, S_-, I_-S_z, I_zS_-\}$

$+1: e^{-iF_z\phi}\{I_+, S_+, I_+S_z, I_zS_+\}e^{iF_z\phi} = e^{-i\phi}\{I_+, S_+, I_+S_z, I_zS_+\}$

$\pm 2: e^{-iF_z\phi}\{I_\pm S_\pm\}e^{iF_z\phi} = e^{\mp i2\phi}\{I_\pm S_\pm\}$

Thus, if we expand the state ρ_t for $\phi = 0$ in terms of groups of operators G_p characterized by a certain coherence order p:

$$\rho_t(\phi=0) = \sum_{p=-2}^{2} G_p$$

: for a pair of spins I-S with

sum of coherences

$p = -2, -1, 0, +1, +2$

$G_0 = a\cdot\mathbb{1} + b\cdot I_0 + c\,S_0 + d\,I_0S_0 + e\,I_+S_- + f\,I_-S_+$ zero effect

$G_1 = g\,I_+ + h\,S_+ + i\,I_+S_0 + j\,I_0S_+$ one raising of

$\vdots$ recall: delays & coils where

Then, the density matrix $\rho_t(\phi)$ that will be obtained by shifting the phase of all the pulses in the sequence by ϕ will be:

$$\rho_t(\phi) = \sum_{p=-2}^{2} G_p \cdot e^{-ip\phi}$$

Now we know what phase cyc does w/ orth. Does not

Summary

Even when dealing with complex pulse sequences, the evolution of coherence orders G_p becomes very simple

i) It always starts with $G_0 = I_z + S_z$

ii) The first pulse can only make G_{+1}, G_0 or G_{-1}

iii) During free evolution (in the presence of chemical shifts or J-couplings) the order of all the G_p is conserved

iv) If we have N coupled spins, an arbitrary second rf pulse can create **all** coherent states ranging from +N to -N, starting from the G_{+1}, G_0, G_{-1} created by the first rf pulse

v) Phase shifting all pulses by ϕ transforms each G_p into $G_p e^{-ip\phi}$

This means that we go from

when $\phi = 0$

$$\rho_0 = \sum_{p=-N}^{N} G_p$$

to

$$\rho(\phi) = \sum_{p=-N}^{N} G_p \cdot e^{-ip\phi}$$ upon phase shifting

This expression, which looks like a Fourier transform, tells us how it would be possible to follow the evolution of a particular coherence order G_p.

Let's assume that we can detect the signals S arising from the time evolution of **all** the coherences. Then, by carrying out M = 2N + 1 phase-shifted experiments and combining the signals according to

$$G_p = \sum_{k=1}^{M} S(\phi_k)\, e^{ip\phi_k} \quad ; \quad \phi_k = \frac{2\pi(k-1)}{M} \quad ; \quad k = 1, 2, \ldots, M$$

we could select any **order p**. Demonstration:

* You have 2N coh's ∴ need 2N+1
* If less then get FOLDING

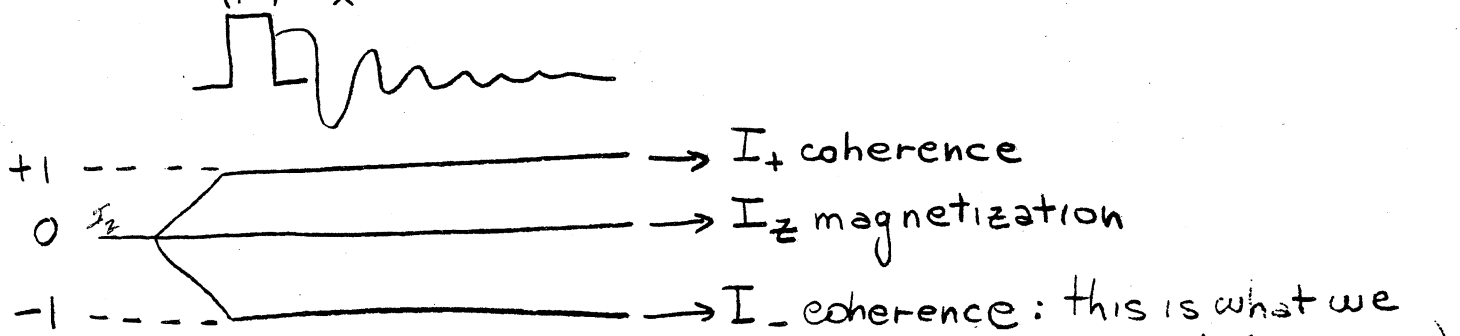
$$\sum_{k=1}^{M} s(\phi_k)\, e^{ip\phi_k} \propto \sum_{k=1}^{M} \sum_{p'=-N}^{N} G_{p'}\, e^{ip\phi_k} e^{-ip'\phi_k} \qquad \text{Proof}$$

$$= \sum_{p'=-N}^{N} G_{p'} \underbrace{\sum_{k=1}^{M} e^{-i2\pi(p'-p)\cdot(k-1)/(2N+1)}}_{\neq 0 \text{ for } p'=p \;;\; 0 \text{ otherwise}}$$

$$\Rightarrow \quad \sum_{k=1}^{M} s(\phi_k)\, e^{ip\phi_k} = G_p$$

Consider as an example the simplest system: an isolated spin 1/2 subjected to an rf pulse $(\beta)\varphi_{Tx}$

1st pulse can only excite these 3



$$\rho(\varphi_{Tx}=0) = \cos\beta \cdot I_0 + \sin\beta\, e^{-i\omega t} I_+ + \sin\beta\, e^{i\omega t} I_-$$

(equilibrium; how they evolve)

If we now make an experiment with $\varphi_{Tx}=\pi$ (change transmitter phase)

$$\rho(\varphi_{Tx}=\pi) = \cos\beta \cdot I_0 + \sin\beta \underbrace{e^{i\pi}}_{-1} e^{-i\omega t} I_- + \sin\beta \underbrace{e^{i\pi}}_{-1} e^{i\omega t} I_+$$

($e^{i\pi}$: phase cycling)

$\Rightarrow \rho(\varphi_{Tx}=0) + \rho(\varphi_{Tx}=\pi) : I_0$

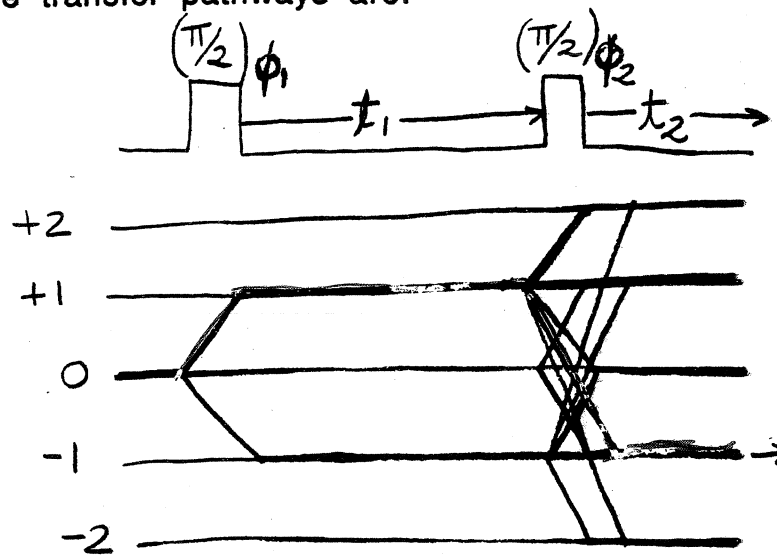
$\rho(\varphi_{Tx}=0) - \rho(\varphi_{Tx}=\pi) : I_+, I_-$ (reversed)

: **This is what we normally do by software**

VI.6 PHASE CYCLING IN 2D NMR

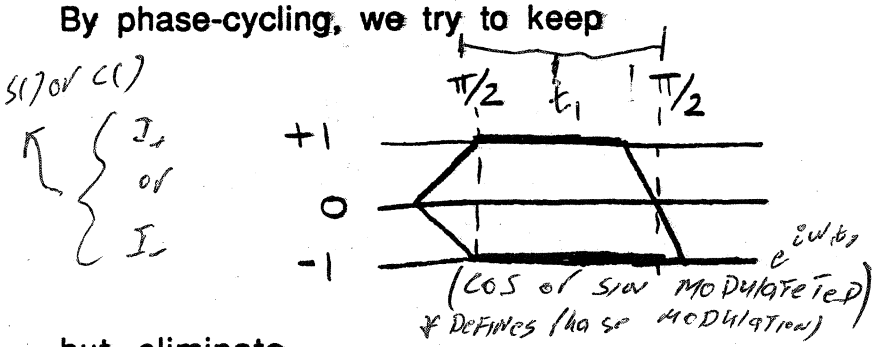
Since free evolution after a pulse just consists of certain orders of coherence, a complete 2D NMR experiment is described by **coherence transfer pathways**; at the end of the experiment however, we can only **detect** the -1 coherence pathway: we can only see $Tr(\rho \cdot I_+)$. Thus, to understand the effects of shifting the rf pulses during a pulse sequence, we have to consider its effects **on each of the different coherence pathways finishing in -1.**

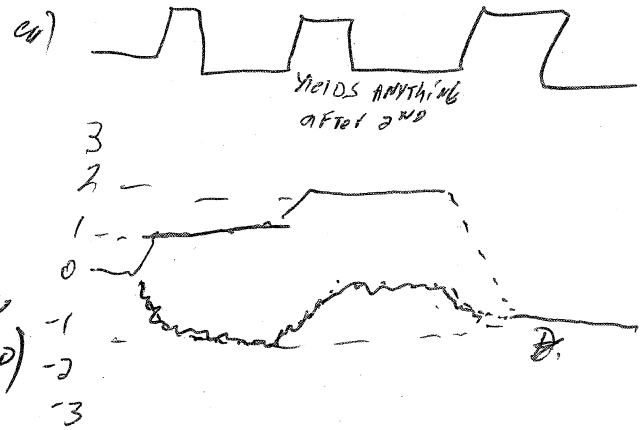
If we consider the complete 2D COSY NMR experiment for instance, and we assume to have only pairs of coupled spins (i.e., $N_{max} = 2$), all the possible coherence transfer pathways are:



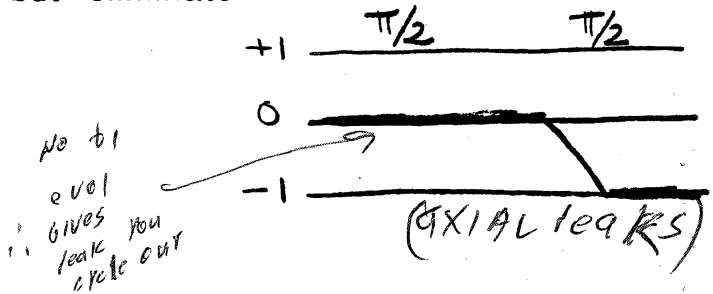
only detect signal at -1 due to coil

By phase-cycling, we try to keep





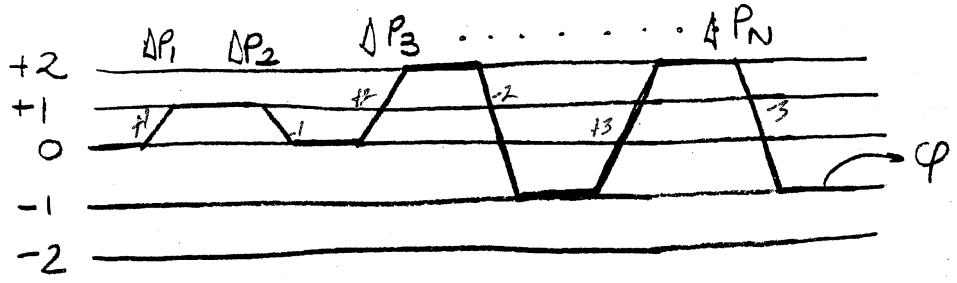
but eliminate



But phase cycle out this one or that one to History of evolution
* Change Phase of Pulses

Double quantum filter ∴ yields all absorptive peaks

In general, if we have an arbitrary pulse sequence with several pulses, very complex coherence transfer pathways can result:



$\Delta P_1 = +1$
$\Delta P_2 = -1$
$\vdots$
$\Delta P_N = -3$

$\vec{\Delta P}$: vecto
characterizi
the coherenc
transfer
pathway

If the rf phase of pulse i is changed by $\Delta\varphi_i$, the density matrix $\rho(\vec{\Delta P})$ changes by a phase factor $\exp(-i\,\Delta\varphi_i\,\Delta P_i)$

* how change phase.
* what matters is rel. change of phase order

For a particular experiment of an arbitrary phase cycling scheme, we can group the rf phase shifts of all the pulses in a vector $\vec{\Delta\varphi}$:

$$\vec{\Delta\varphi} = (\Delta\varphi_1, \Delta\varphi_2, \ldots, \Delta\varphi_N)$$ = how much your changing phase of a particular pulse

and express the final density matrix $\rho(\vec{\Delta\varphi})$ in terms of the density matrices that would arise from the different pathways in the absence of phase shifts as

Density matrix changes this much $\vec{\Delta P}\cdot\vec{\Delta\varphi}$

Gains this phase factor a#!

$$\rho(\vec{\Delta\varphi}) = \sum_{\vec{\Delta P}} \rho(\vec{\Delta P}, \vec{\Delta\varphi}=0)\cdot\exp(-i\cdot\vec{\Delta P}\cdot\vec{\Delta\varphi})$$

looks like $\mathcal{F}$ FT

state arising from a coherence transfe pathway $\vec{\Delta P}$ in the absence of any phase shifting. (INIT CONDITION

Fish out, set of of transmitter to this.

Suppose we want to select a particular $\rho(\vec{\Delta P})$ using a series of phase-cycled experiments. We can eliminate other coherence transfer pathways by recalling from Section II the fact that the signal detected in an NMR experiment also depends on the phase of the receiver:

$$S(t) \propto \mathrm{Tr}(\rho^{(t_1,t_2)} F_+)\, e^{i(\varphi_{Tx}-\varphi_{Rx})}$$

In the present case, the NMR selection rule only allows us to detect the coherence pathway which finishes at p = -1. In other words, the particular $\rho(\overrightarrow{\Delta p})$ that we can select has to fulfill $T_R(\rho F_+) \equiv$ starting at zero and ending at -1:

$$\sum_{i=1}^{N} \Delta p_i = -1$$

N- components must fulfill this & corresponds to prev. page

We can now use the behavior of the signal arising from this coherence upon phase cycling:

$$S(t) = \rho(\overrightarrow{\Delta p}, \overrightarrow{\Delta\varphi}=0)\, e^{i\underbrace{(-\overrightarrow{\Delta p}\cdot\overrightarrow{\Delta\varphi})}_{\varphi_{Tx}}}\, e^{-i\varphi_{Rx}}$$

The condition for adding up this signal coherently throughout the phase-cycled experiments will therefore become

$$-\overrightarrow{\Delta p}\cdot\overrightarrow{\Delta\varphi} - \varphi_{Rx} = 0$$

Fulfill this for each scan

$$\Longleftrightarrow \quad \boxed{\varphi_{Rx} = -\sum_{i=1}^{N} \Delta p_i \cdot \Delta\varphi_i}$$

Recipe for phase cycling

Note: keep in mind that when phase shifting for each pulse in steps of

$$\Delta\varphi_i = 2\pi/k_i$$

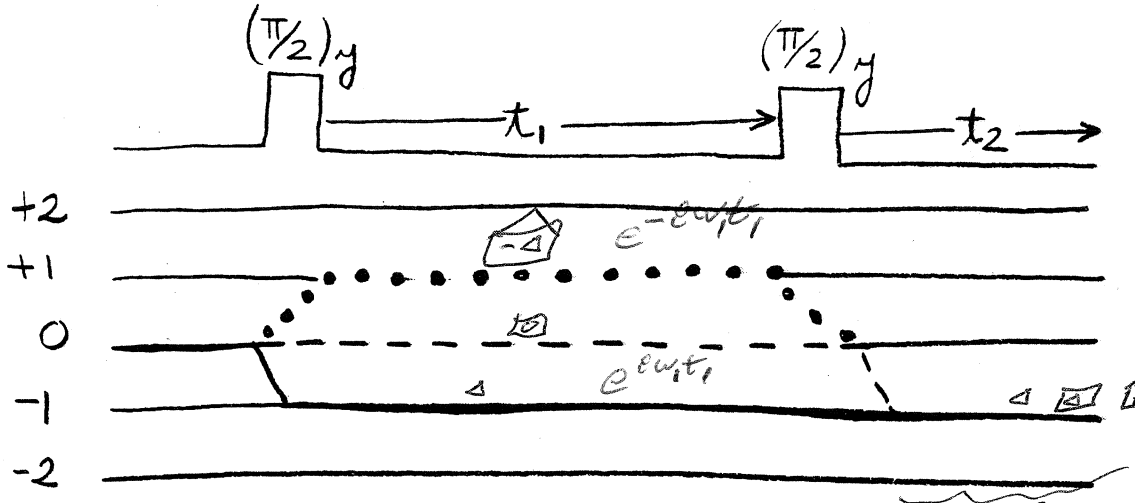
$$\varphi_k = \sum_{k=1}^{M} \frac{2\pi(k-1)}{M}$$

we let a **manifold** of coherences through: $p_i, p_i \pm k_i, p_i \pm 2k_i$, etc.

Know Δp & $\Delta\varphi$ Then know what to set receiver Phase (swalling real & Im)

Phase cycle Defn? How do you change phase of c & M. ON Final. Figure out from Recipe

Let's consider once again the 2-pulse COSY. There are 3 coherence transfer pathways which may eventually contribute to the observed signal:



RMS MODEL (+1 & -1 PATHS) $\phi = 0, \pi$ DISTINGUISH

Let Δ = Press Free

Defines Krauser Paths

—— : $\Delta p_1 = -1$, $\Delta p_2 = 0$: p-type or anti-echo-type pathway

– – – : $\Delta p_1 = 0$, $\Delta p_2 = -1$: axial signal pathway

•••• : $\Delta p_1 = +1$, $\Delta p_2 = -2$: n-type or echo-type pathway

∴ uniquely defined by Δp's

Antiechoes give signals of the type $\exp(i\omega_1 t_1)\exp(i\omega_2 t_2) \rightarrow$ peak at (ω_1, ω_2)

Echoes give signals of the type $\exp(-i\omega_1 t_1)\exp(i\omega_2 t_2) \rightarrow$ peak at $(-\omega_1, \omega_2)$

Axial pathways give signals of the type $\exp(i\omega_2 t_2) \rightarrow$ peak at $(0, \omega_2)$

These are the 3 type of peak one gets without phase cycling. To get rid of axial peaks it is enough to retain the echo and antiecho pathways. To get also quadrature detection in t_1 however, we have to distinguish among all these pathways. In either case, we achieve the goal by phase-cycling. We therefore write the pulse sequence as

$$(\pi/2)_{y+\phi_1} - t_1 - (\pi/2)_{y+\phi_2} - t_2$$

Just for t_1 times

Negative times

Positive times

t_1

t_2

$\therefore \Delta p_1 = +1, \Delta p_2 = -2$

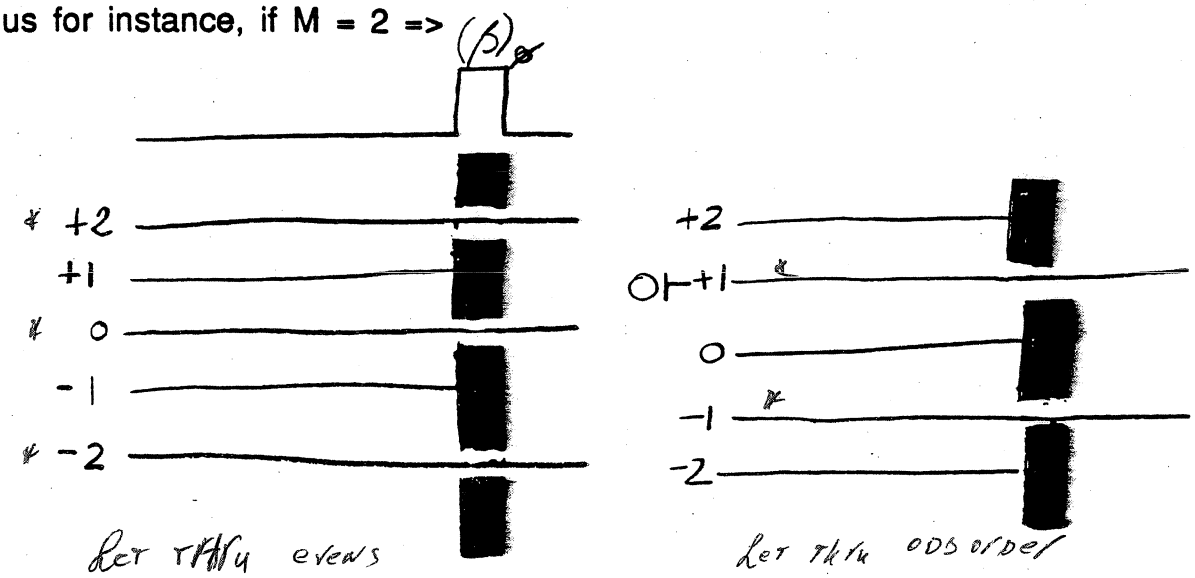
Antiechoes

Small signal

New (based on Folding Arguments)

In general, phase cycles act as a **mask**: they only leave certain **orders** of coherences. By phase cycling ϕ M times between 0 and 2π, one can select coherences whose orders are $p(\beta) \pm k \cdot M$; $k = 0, 1, 2, \ldots$ * mask = suppress orders of coh.

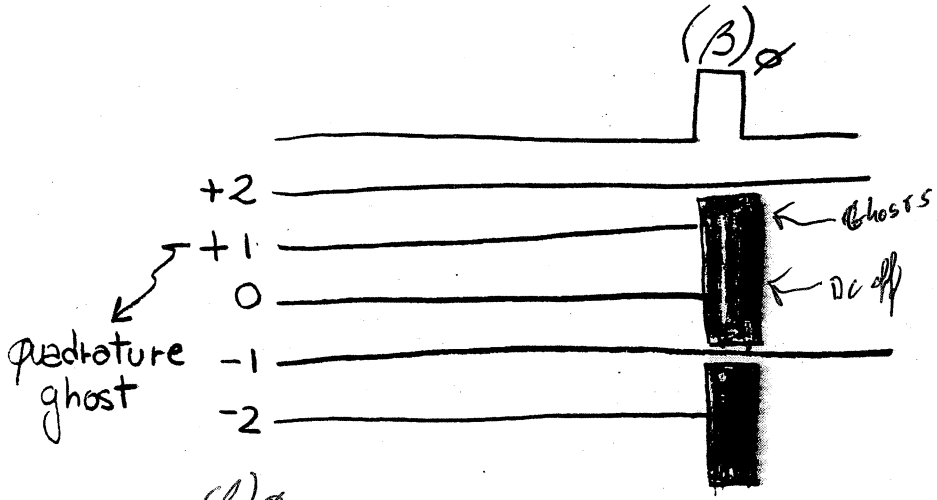
Thus for instance, if M = 2 =>



* Let thru depends on what you set Rec Phase too (adding & sub)

Uncoupled spins

It is now evident why phase cycling the excitation pulse by ϕ = 0, $2\pi/3$, $4\pi/3$, is enough for eliminating quadrature ghosts in uncoupled spin systems, where ±1 are the maximum orders of coherences.



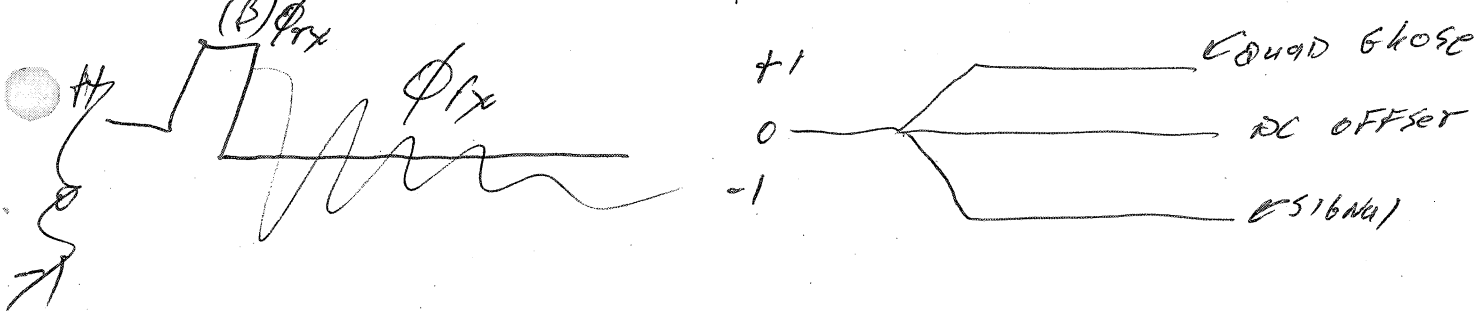
$\frac{2\pi}{3} = \phi_{Tx}$

elim 1, need 2

" 2, " 3 =

M = 3

$c(\phi_{Tx})iR - c\overline{iR}\,s(\phi_{Tx})$



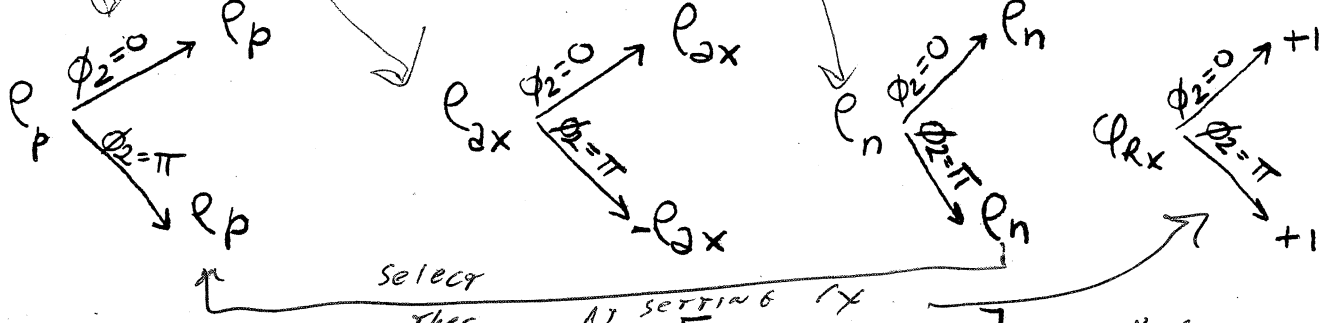
We start by keeping $\Phi_1 = 0$ fixed (normally this pulse is also phase-cycled in order to eliminate quadrature ghosts in Φ_2). The behavior of the different signals with respect to Φ_2 :

(Positive) $\rho_p \longrightarrow \rho_p$ no change

$\rho_{axial} \longrightarrow \rho_{axial} \cdot e^{i\Phi_2}$

$\rho_N \longrightarrow \rho_N \cdot e^{2i\Phi_2}$

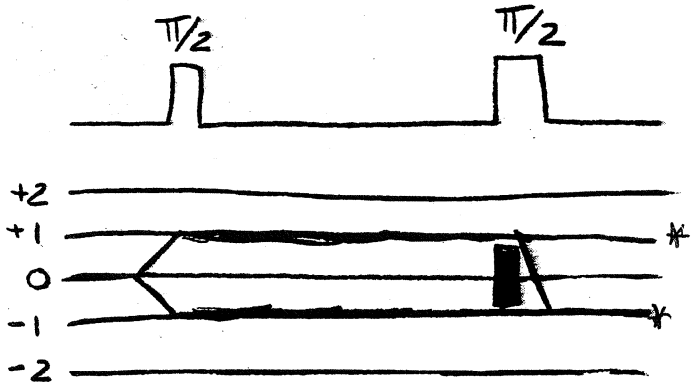
A phase cycling $\Phi_2 = \{0, \pi\}$ can eliminate the axial peaks but not the quadrature image:



$$\Rightarrow S(t) = S_0 + S_\pi \propto Tr\left[(\rho_p + \rho_n)\cdot F_+\right]$$

* removes Axial peak, still AMPLIT MODulated

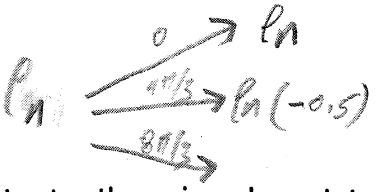
The "mask" of this phase cycling:



* Allows Now For Quad Detection

Can Detect -1 or +1 Really.

3 cycles for Antiecho



To eliminate the signals arising from both ρ_{ax} and ρ_N, $\phi_2 = 0, 2\pi/3, 4\pi/3$ is the minimum phase cycling that can be used.

The phases of the different transfer pathways and of the R_x in the phase cycling:

$C(\frac{2\pi}{3}) = -0.5$

$S(\frac{2\pi}{3}) = 0.866$ use (-) in k_x to compensate for (+)'s

$C(\frac{4\pi}{3}) = -0.5$

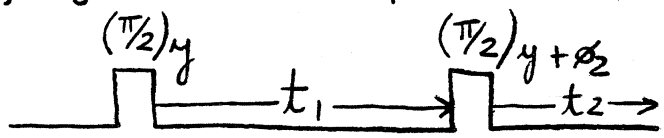
$S(\frac{4\pi}{3}) = -0.866$

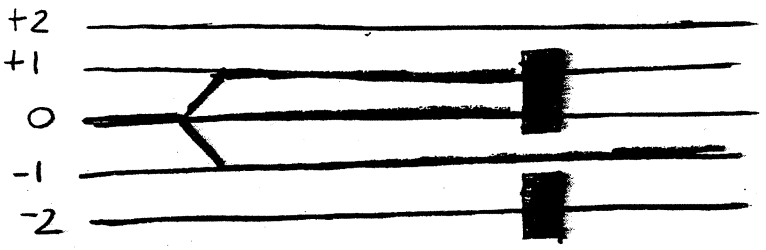
	echo $\Delta p_2 = -2$	axial $\Delta p_2 = -1$	antiecho $\Delta p_2 = 0$	ϕ_{R_x}
$\phi_2 = 0$	ρ_n	ρ_{AX}	ρ_p	0
$\phi_2 = 2\pi/3$	$\rho_n e^{+i4\pi/3}$	$\rho_{AX} e^{+i2\pi/3}$	ρ_p	0
$\phi_2 = 4\pi/3$	$\rho_n e^{+i8\pi/3}$	$\rho_{AX} e^{+i4\pi/3}$	ρ_p	0

The signals arising from the echo and the axial coherence transfer pathways can be eliminated by adding the signals from the three experiments. The effects of such a phase cycling can therefore be pictured as

$-(\frac{8\pi}{3}) = -0.5$

$S(\frac{8\pi}{3}) = -0.866$





but have probs. of R→+1 I→+2

$C(\frac{2\pi}{3})$ electrons don't like this!

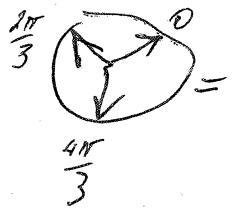
IF change phase of $\phi_{R_x} = \frac{2\pi}{3}, \frac{4\pi}{3}$ ∴ get ONLY echo signal!

Recall $\phi_{R_x} = -\sum \Delta p_e \Delta\phi_e$

$\Delta p_2 = -1$ | $\Delta_2 = 0$

echo: ∴ $\phi_{R_x} = 0$

AXIAL



= ONLY see ANTIecho

Increments of $2\pi/3$ in the phase of the rf are not common in NMR spectrometers, but increments of $\pi/2$ are. Therefore, selection of the antiecho pathway is usually achieved using $\phi_2 = 0, \pi/2, \pi, 3\pi/2$. A common notation for these quadrature phases is

$\phi_2 = 0 \longrightarrow 0$ (or x); $\phi_2 = \frac{\pi}{2} \longrightarrow 1$ (or y); $\phi_2 = \pi \longrightarrow 2$ (or -x)

$\phi_2 = \frac{3\pi}{2} \longrightarrow 3$ (or -y)

* MULTIPLES OF $\pi/2$ * WAY ARE MACHINES ARE SET UP

Data from these experiments can be used to select the signal arising from either the echo, the axial, or the antiecho pathways, due to the fact that they gain a different phase as a function of ϕ_2:

add R&II, swap R&II,
0 1 2 3

	echo	axial	antiecho
$\phi_2 = 0$	0	0	0 add
$\phi_2 = 1$	2	1	0 add
$\phi_2 = 2$	$4 \equiv 0$	2	0 add
$\phi_2 = 3$	$6 \equiv 2$	3	0 add

i, ϕ_{1+} add & subt, add, subt

Where we used the fact that the phase is periodic modulus 4 .

The phases of the receiver ϕ_{Rx} again follow from the basic equation

$$\phi_{Rx} = -\Delta p_1 \cdot \phi_1 - \Delta p_2 \cdot \phi_2$$

$$= -\Delta p_2 \cdot \phi_2 \quad \text{, assuming that } \phi_1 \text{ is kept constant (i.e. at 0}$$

By setting the appropiate ϕ_{Rx} , one can therefore select

e → Hobley

$$e_p = S(0) + S(1) + S(2) + S(3)$$
$$e_{ax} = S(0) - iS(1) - S(2) + iS(3)$$
$$e_n = S(0) - S(1) + S(2) - S(3)$$

S(0), S(1), ...: signe from $\phi_2 = 0, 1, \ldots$

Recall from what we saw in Section II·10 , that the data rearrangement implied by the $e^{i\phi_{Rx}}$ factor can be done by software processing. The selection of the peak from the axial coherence pathway for instance

$\phi_2 = 0 : \phi_{Rx} = 0 \Rightarrow$ ADC1 → Buffer 1 (R); ADC2 → Buffer 2 (I)

$\phi_2 = 1 : \phi_{Rx} = \pi/2 \Rightarrow$ -ADC2 → Buffer 1 (R); ADC1 → Buffer 2 (I)

$\phi_2 = 2 : \phi_{Rx} = \pi \Rightarrow$ -ADC1 → Buffer 1 (R); -ADC2 → Buffer 2 (I)

$\phi_2 = 3 : \phi_{Rx} = \frac{3\pi}{2} \Rightarrow$ ADC2 → Buffer 1 (R); -ADC1 → Buffer 2 (I)

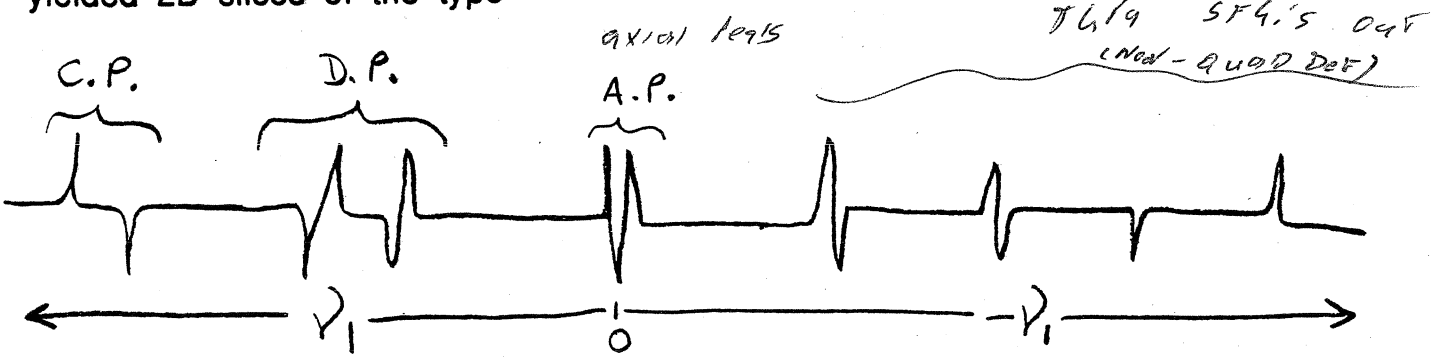
Now we've managed to achieve Quad Det in 2D NMR
" suppress axial leaks
& Also achieved time Resolution in t1 also

VI.7 PURELY-ABSORPTIVE LINE SHAPES IN 2D NMR

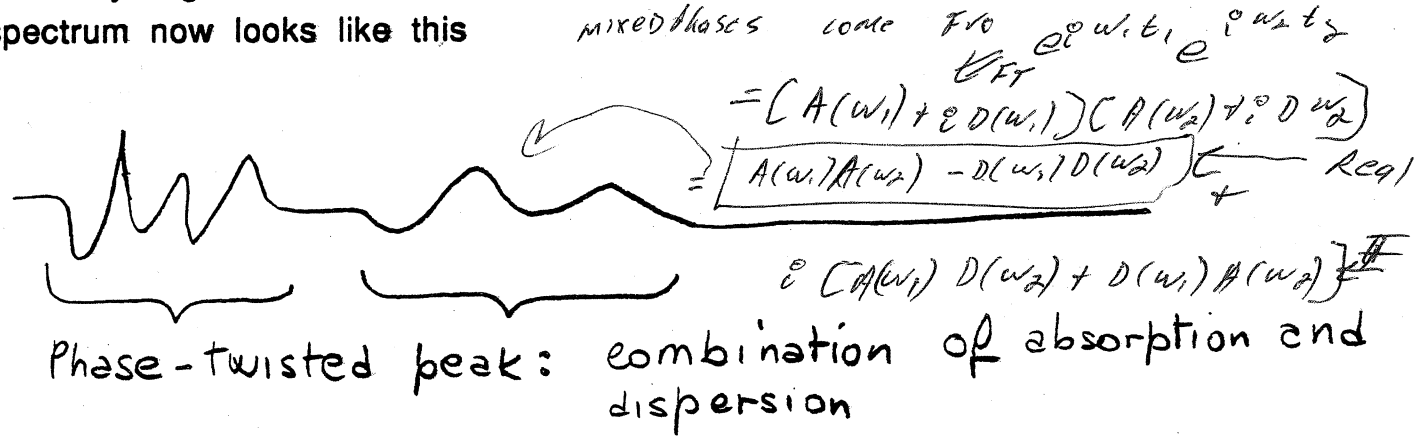
Our initial 2D COSY NMR experiment

$$(\pi/2)_y - t_1 - (\pi/2)_y - t_2$$

yielded 2D slices of the type



Elimination of the axial peak and quadrature detection along ν_1 were achieved by phase-cycling out the axial- and echo-coherence pathways. Unfortunately, our spectrum now looks like this



We got these infamous line shapes due to the fact that:

we went from $\sin(\omega_1 t_1)\, e^{i\omega_2 t_2}$: amplitude modulation; non-quadrature

to $e^{i\omega_1 t_1}\, e^{i\omega_2 t_2}$: phase modulation; quadrature

In general, in order to get reasonable line shapes in 2D NMR experiments recorded using quadrature t_1-detection, one has to

i) either take the magnitude of the spectrum and loose a lot of resolution, or

ii) get a purely-absorptive spectrum, an approach which requires the acquisition of a 2nd 2D time-domain set.

The reason why purely-absorptive experiments are worthwhile

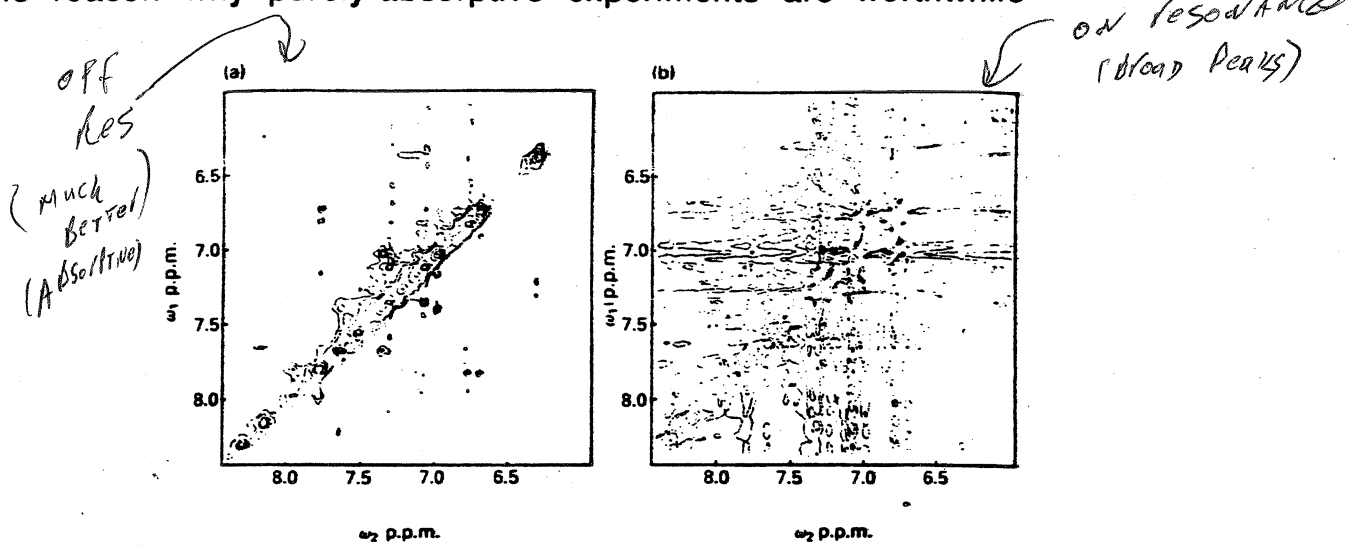


FIG. 6.5.9. Comparison of 2D spectra presented in pure 2D absorption mode (a) and in absolute-value mode (b), illustrating the advantage of pure phase spectra for enhancing resolution. These 2D NOE spectra from the protein basic pancreatic trypsin inhibitor show only the aromatic region. Both spectra were computed from the same data, with the same Gaussian filtration, and the contour levels are drawn at 0.15, 0.3, 0.6, 1, 2.5, 5, and 10 per cent of the maximum peak. (Reproduced from Ref. 6.28.)

Have Quad Det Now, But No Purely Absorptive

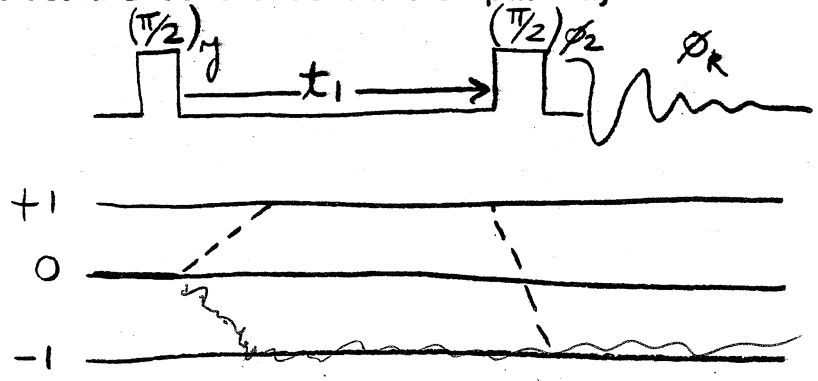
As mentioned before, the key to purely-absorptive line shapes lies in obtaining an echo along t_1 <=> collect both the echo and antiecho coherence pathways.

To Get Both want to run

$S(t_1, t_2)$ = echo } run independently (if not sum of 2)

$S(t_1, t_2)$ = anti " " }

The 2 experiments that can afford these data differ in the phase cycling they use to select the coherence transfer pathway:

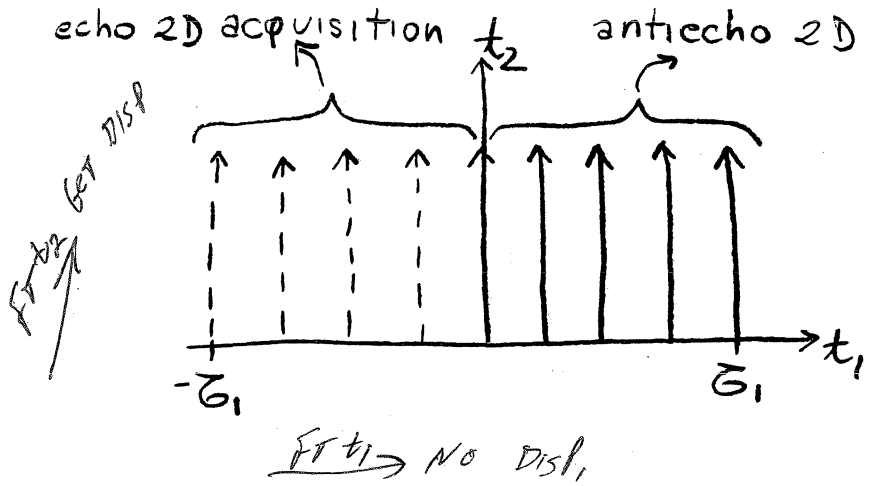


ϕ_2	$\phi_R = -\Delta p \cdot \phi_2$
0	0
1	0
2	0
3	0
0	0
1	2
2	0
3	2

To

2 2D spectra

These acquisitions correspond to the following time-domain sampling:



$s(t_1, t_2) = e^{i\omega_1 t_1} e^{i\omega_2 t_2}$

$s(t_1, t_2) = e^{-i\omega_1 t_1} e^{i\omega_2 t_2}$ ##

SIGN of t_1 changes only

By rearranging the data acquired as shown in this scheme, 2D FT would give a signal

$$S(\omega_1, \omega_2) = S_0 A(\omega_1) \cdot e^{-i\omega_1\tau_1} \cdot [A(\omega_2) + iD(\omega_2)]$$

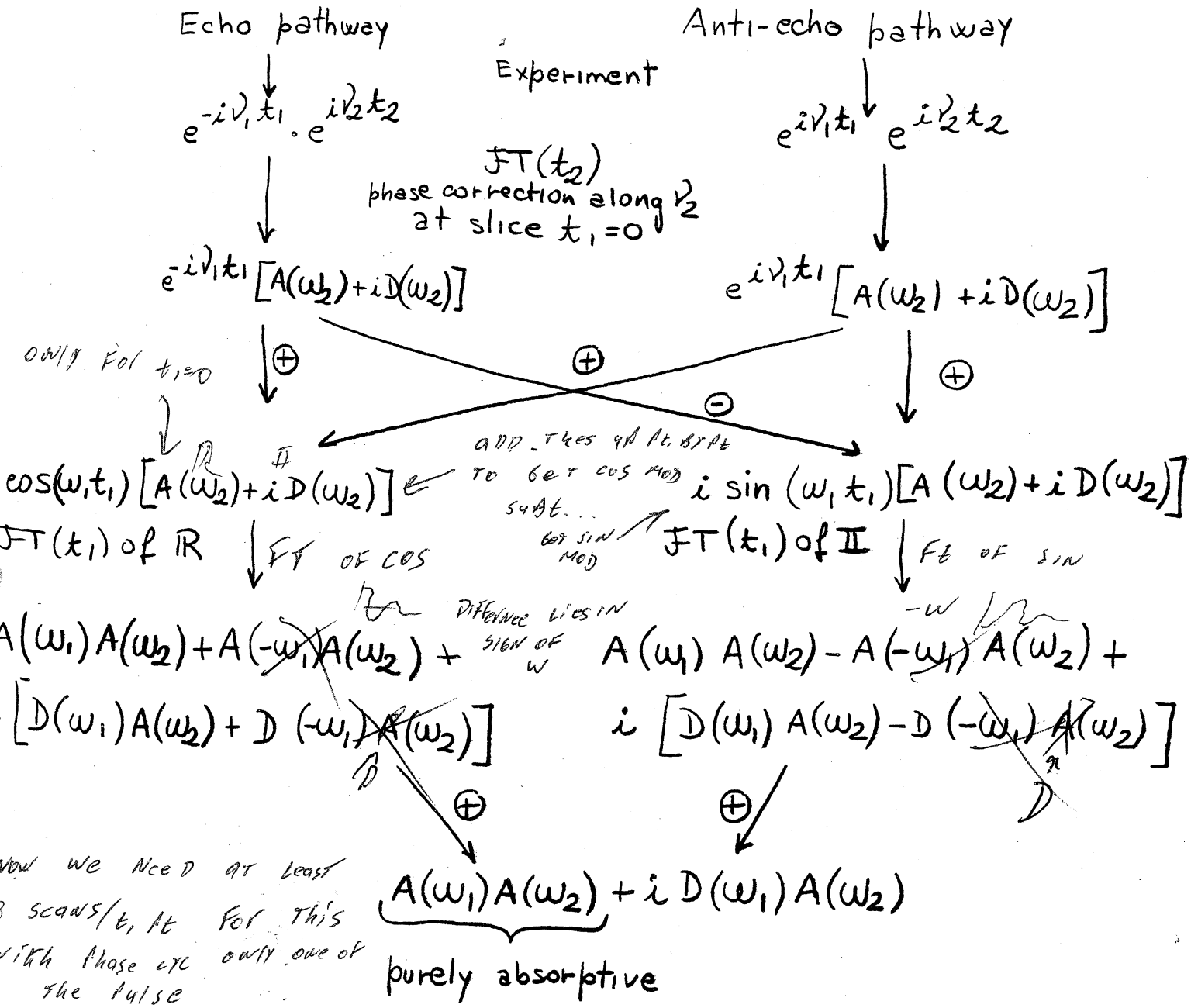
Not purely real, some DISP Due to this
Very large 1st order phase correction.

=> by applying a large first-order phase correction $e^{i\nu\tau_1}$ and keeping the real part only, purely absorptive line shapes $A(\omega_1) \cdot A(\omega_2)$ without the phase twist are obtained.

∴ FT & APPLY large 1st order phase correct & TAKE MAGN

Another method to have A signal

People do not like to apply large first-order phase corrections. Instead, the following strategy is followed:



* Now we need at least 8 scans/t_1 pt for this with phase cyc only one of the pulse

Phase cyc on both pulses ∴ need 32 scans/pt

There is more than one way of processing 2D data that yield to purely-absorptive line shapes. They are all more or less equivalent, and they all require the acquisition of two complementary 2D NMR experiments.

In the case of the 2-pulse H,H-COSY, if we consider that we have to phase-cycle the first pulse too to eliminate quadrature ghosts along ν_2, we end up with a 32 scans phase cycle.

* always have to phase correct along t_2 just as for 1D

0-3 0-3 0-3 0-3
0 1 2 3
0 1 2 3

VI.8 DIGITAL RESOLUTION, SELECTIVITY AND NOISE IN 2D NMR

How long will a 2D NMR experiment take?

In normal 1D solution NMR one uses acquisition times long enough to characterize a peak with ≈ 4 points. Thus, for line widths in the order of 1 Hz

$$\Delta\nu \approx 0.25 \text{ Hz} \Leftrightarrow AT = 4 \text{ sec.}$$

Moreover, in order to observe a ^1H NMR spectrum at, say, 500 MHz, one has to sample ca. 10 ppm. These leads to dwell times

$$DW = \frac{1}{SW} \approx \frac{1}{10 \cdot 500\,Hz} = 200\,\mu s$$

Therefore, the number of data points acquired is approximately

$$NP \approx \frac{4}{200 \cdot 10^{-6}} = 20{,}000 \approx 16\text{-}32\ K$$

Suppose we try to do something similar in a 2D H,H-COSY, where each point along the t_1-domain requires **an independent experiment**. Assuming that each scan takes approximately 4 seconds

=> Experimental time (1 scan/t_1 point) ≈ 16,000 x 4 sec.

Moreover, since the COSY phase cycle requires 32 scans

=> Total experiment time ≈ 16,000 x 4 x 32 sec. ≈ 24 days
Total storage required ≈ 16,000 x 32,000 x 8 = 4 Gbytes/FID

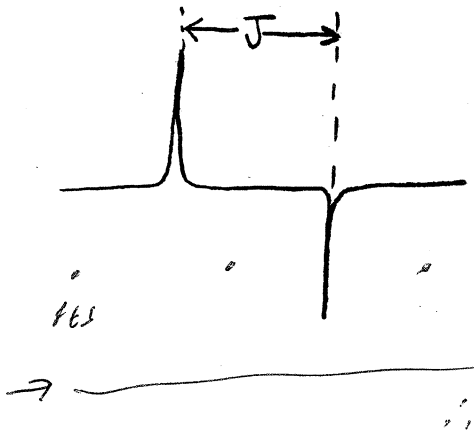
If each experiment would last for so long and take so much space, it would be useless.

6 scans/t_1, ~~also~~ have quad detection 4 x 1915
3 here 3 here

∴ we change resol

The resolution of 1D NMR experiments cannot be extended to 2D NMR

In 2D NMR, line widths no longer determine the acquisition times along either the t_1- or t_2-axis. Instead, acquisition times are determined by the fact that 2D NMR peaks may appear as anti-phase doublets:



Now we at 400
0.25 Hz → 8-5 Hz
(narrowing sw)

∴ J-coal defines resolut

=> if the resolution is poorer than 1 point/J Hz, we will have substantial peak cancellation. Another hint for acquiring 2D NMR spectra is to focus only in the spectral region of interest; one seldom needs to scan 10 ppm for a particular compound. Then assuming J ≈ 5 Hz; 7 ppm @ 500 MHz =>

AT = 1/resol

AT (t_1) = 200 ms; NP (t_1) ≈ 512 or more

$t_t = \#pts \cdot \Delta t$ $\quad \Delta t = 1/sw$

One can be more generous along t_2 as resolution along this dimension is "cheaper" (in terms of acquisition times). Typical parameters would be:

AT (t_2) ≈ 800 ms; NP(t_2) = 2048

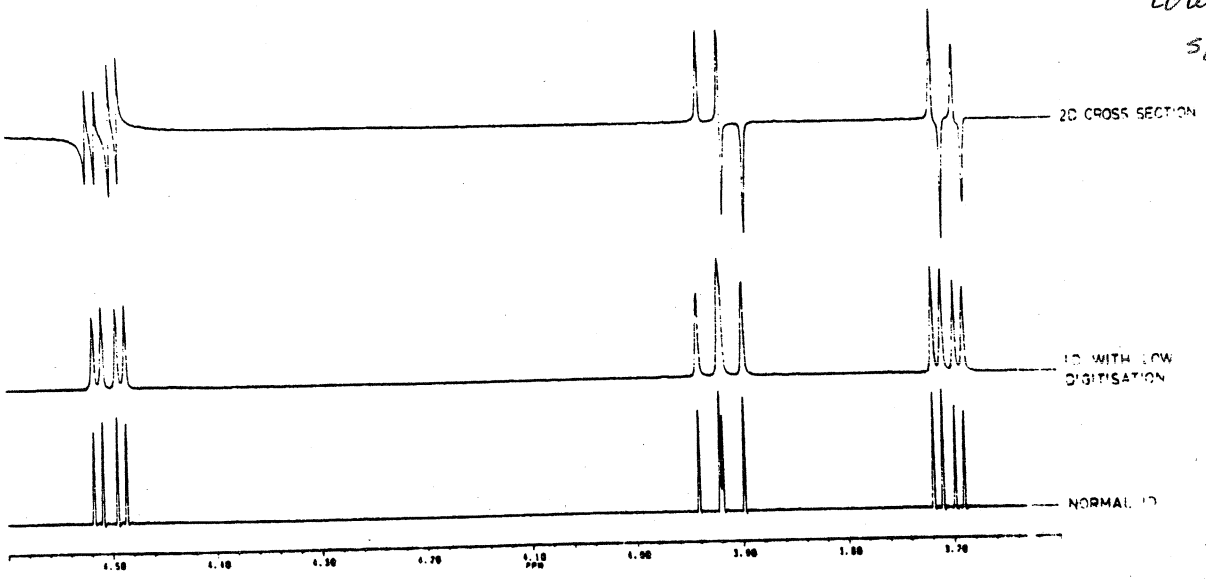
We have then reduced the total acquisition time to ca. 512·32·0.8 s = 3 hs 40 minutes, and the total storage space to 16 Mbytes after zero filling along the t_1 axis.

For purely ABS 3D?

$(A + i\omega_1)(A + i\omega_2)(A + i\omega_3)$

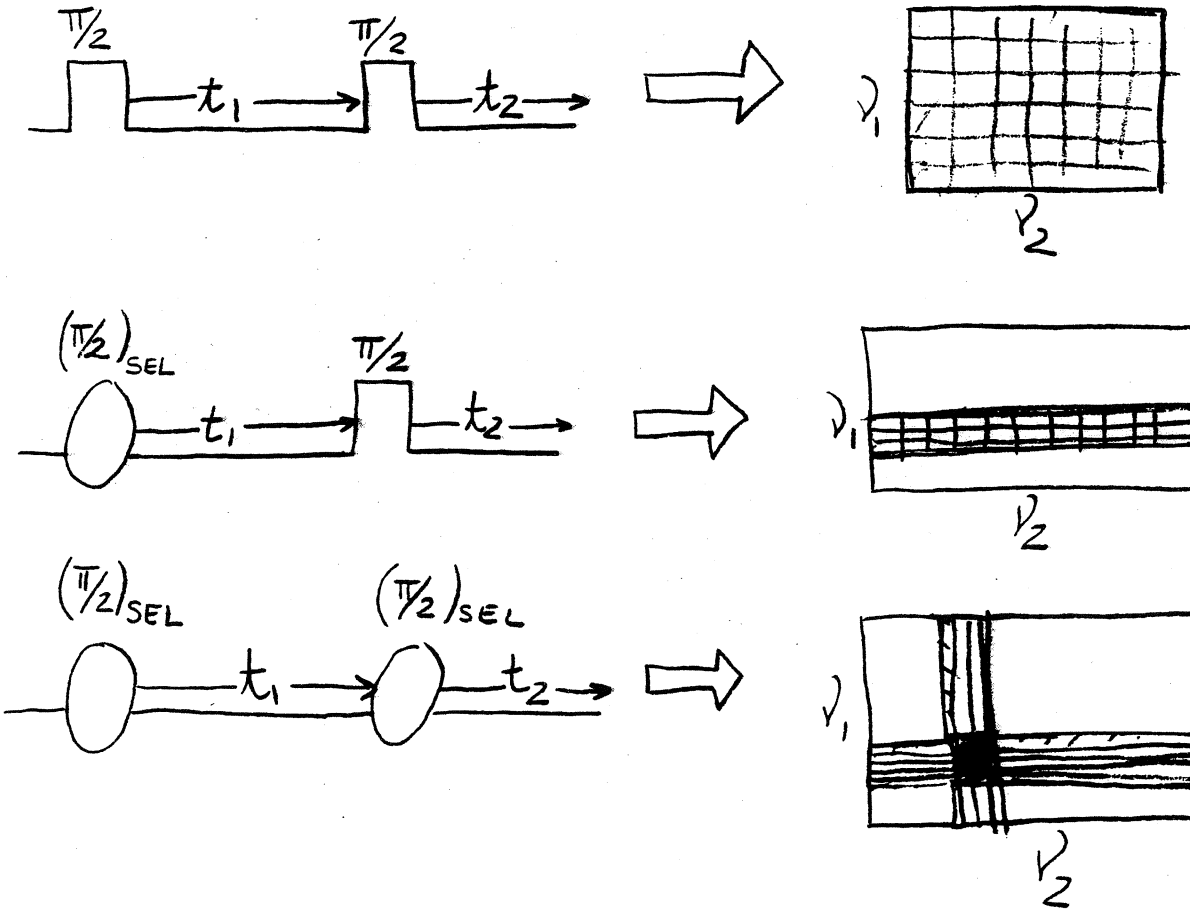
The resolution of such a 2D experiment is still better than the one of a 1D experiment with low digitization:

1D
low resol. spectra

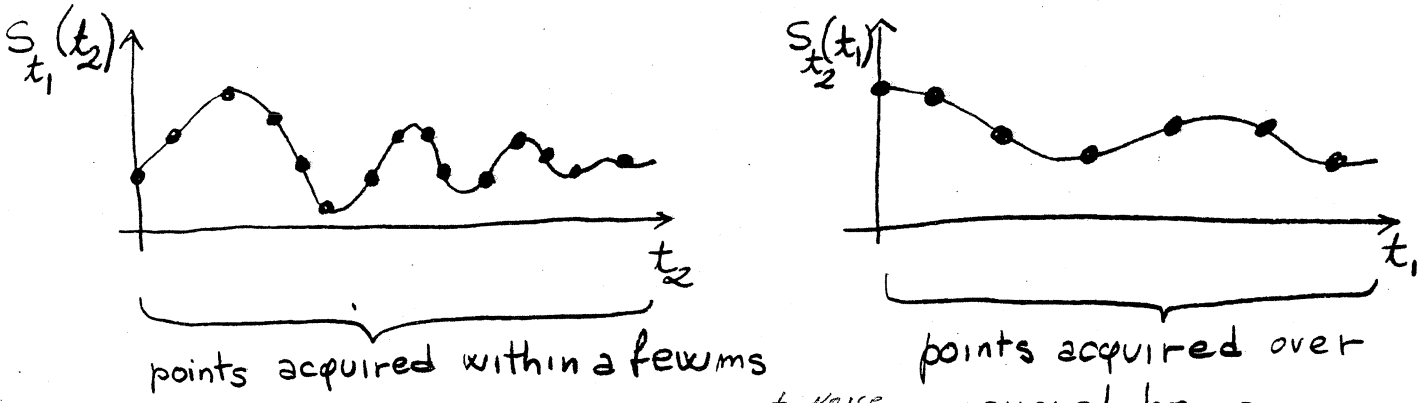


Sometimes high-resolution in 2D NMR is needed for accurate measurement of coupling constants. This can be achieved using selective excitation:

high res in 2D



The origin of **noise** in a 2D FID is not the same as in 1D NMR. In a normal, directly digitized FID, the main source of noise is thermal and originates in the probe; this noise affects $S_{t_1}(t_2)$ for a given t_1. Along t_1 however the main source of noise are instabilities of the instrument over long periods of times:



t_1 noise shifts pts

all peaks have t_1 noise, all suffer from same instrument

t_1- noise is a very serious problem when strong signals (e.g. H_2O) are present:

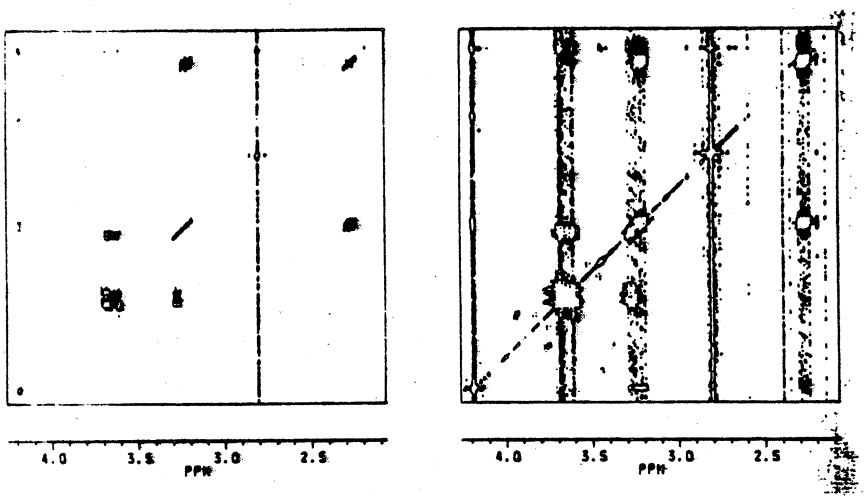


Figure 8.30 With a fairly high minimum contour level (left) a band of t_1 noise is only apparent for the strong signal at 2·8 p.p.m., but by plotting a lower contour (right) it is revealed as being associated with all signals.

t_1 noise an artifact of long term instabilities in machine eg) pts shifted a little

One can try to decrase t_1-noise by using a strong lock signal or by cycling the t_1 intervals used according to

scan #1 at $t_1 = 0, \Delta t_1, ..., n\Delta t_1$
scan #2 at $t_1 = 0, \Delta t_1, ..., n\Delta t_1$

Phase cycling can help remove these t_1 noise problems.
eg) MRI's $\pi/2$, π, π,

When dealing with homonuclear 2D spectra and square data arrays, t_1-noise can be decreased using a software trick called symmetrization: discarding data which is not symmetrically placed along the diagonal:

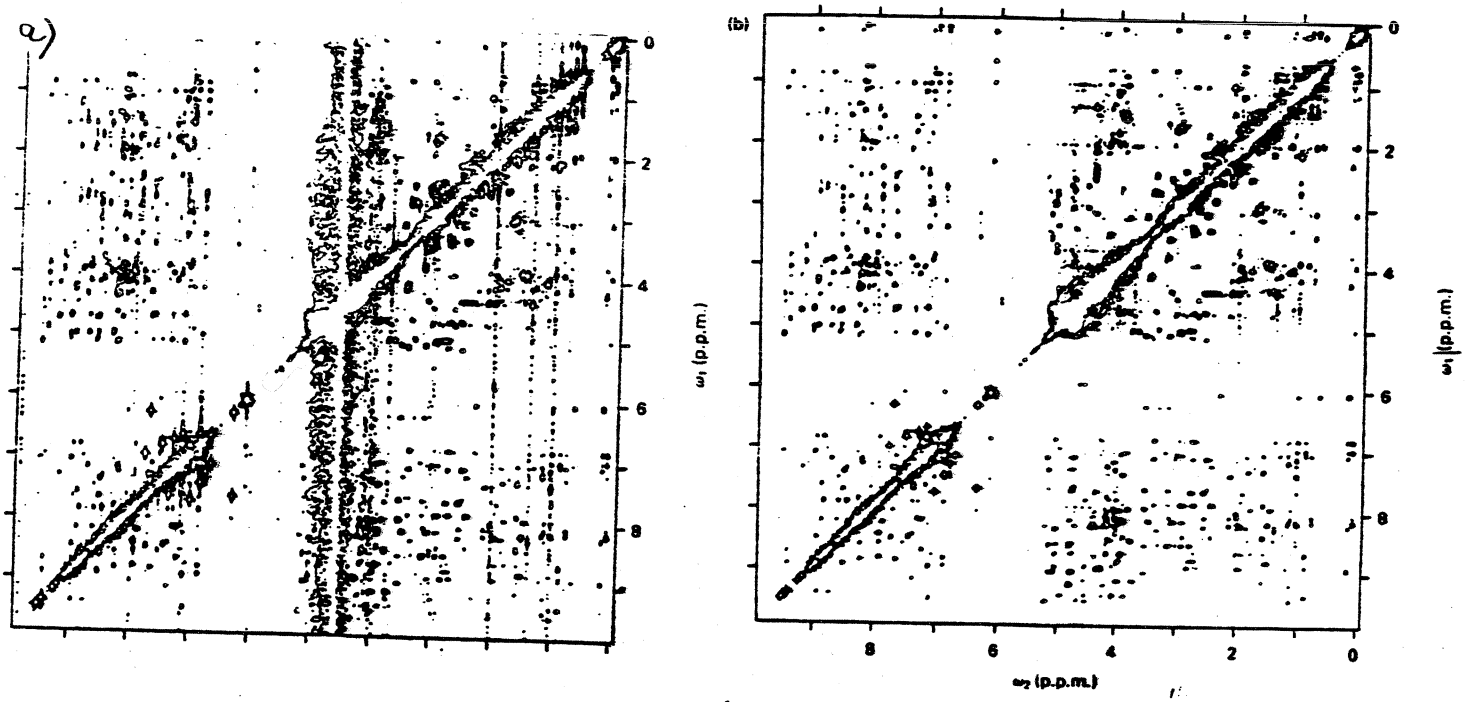


FIG. 6.6.5. The effect of symmetrization, eqn (6.6.16), in an absolute-value 2D NOE spectrum of the protein bull seminal inhibitor II A (molecular mass ≈6500) in H_2O, obtained with the sequence $\pi/2 - t_1 - \pi/2 - \tau_m - \pi/2 - t_2$ with a mixing time τ_m = 200 ms. (a) Original spectrum; (b) symmetrized spectrum. Note the prominent t_1-noise ridges in (a), particularly the artefacts in the vicinity of $\omega_2 = 4.6$ p.p.m. which stem from the H_2O resonance. (Reproduced from Ref. 6.43.)

Although symmetrized spectra look nicer, they may lead to artificial peaks. Beware!

VI.9 GRADIENT ENHANCED SPECTROSCOPY

Ref Quad Echo lines MANY less scans ST

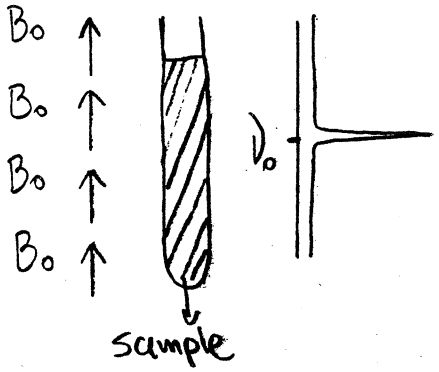
Phase cycling allowed us to obtain purely absorptive line shapes and to eliminate axial peaks. Because it is a difference method, however, it has some disadvantages:

i) always needs several scans, even if S/N is not a problem

ii) even a very small error in the cancellation of large signals (e.g., in H_2O supression) leads to large t_1-noise

There is a way of eliminating these problems which achieves an almost ideal selection of arbitrary coherence transfer pathways in a single scan. It involves the use of **pulsed magnetic field gradients**

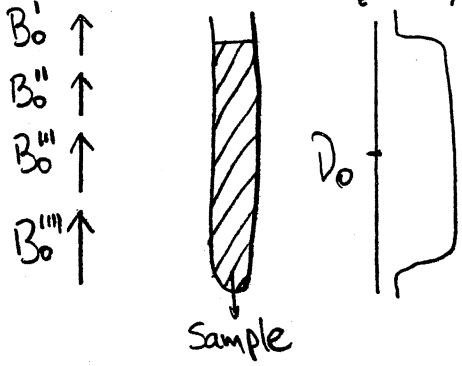
A spectrum in a homogeneous B_0



Rotating frame frequency = 0

Upon adding a linear magnetic field gradient

$B = B_0 + G_z \cdot z$; $G_z = \frac{\partial B_z}{\partial z}$

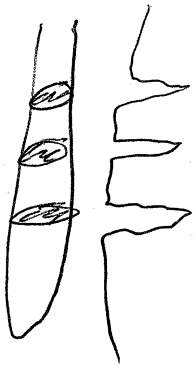


Rotating frame frequency = $\gamma G_z \cdot z$

This is a spatially-dependent phase

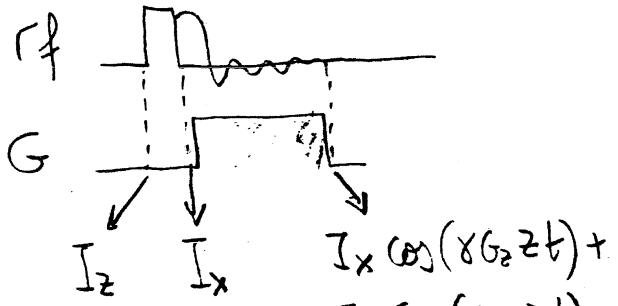
Takes INTO Freq units

eg)



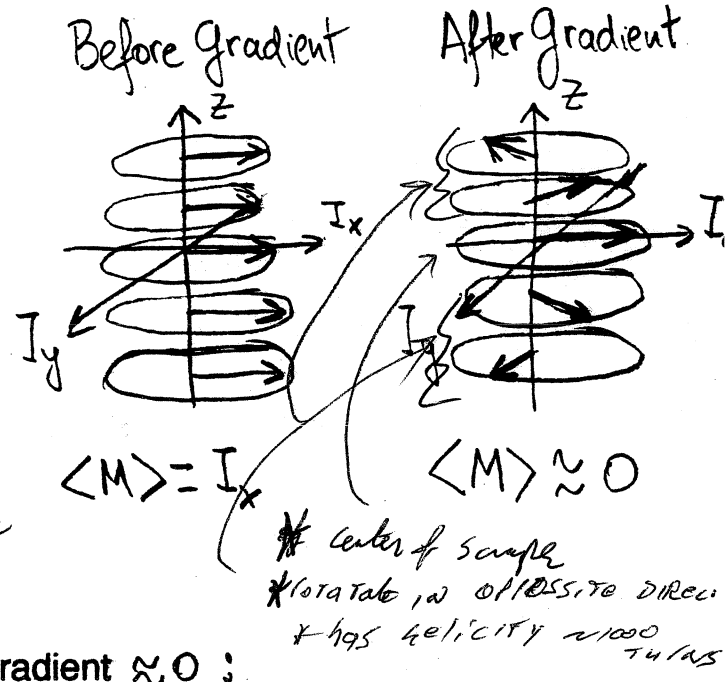
maps 1:1 density of H_2O in sample

Upon applying a gradient pulse after excitation:



hard

like applying c.s. dependent on z



The net magnetization at the end of the gradient ≈ 0 :

$$\langle M_x(t) \rangle = \frac{1}{l} \int_{-l/2}^{l/2} \cos(\gamma G_z z t)\, dz = \frac{\sin\left(\frac{\gamma G l t}{2}\right)}{\left(\frac{\gamma G l t}{2}\right)} = \text{sinc}$$

l: length of sample

Typical values: $l \approx 1$ cm, $G \approx 50$ kHz/cm, $t \approx 3$ ms $\Rightarrow M_x \approx 10^{-3}$-$10^{-4}$

The signal is almost completely gone!

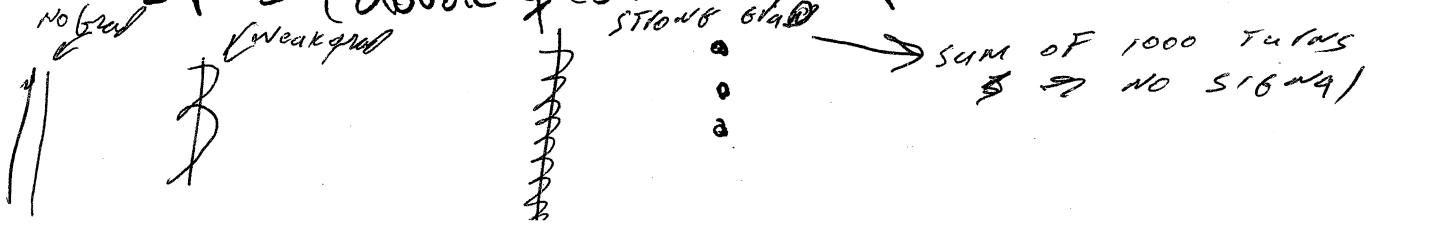
In general, pulse gradients are shaped:  , not 

hard to apply ∴ change here

This behavior can be generalized to the case of a p-quantum coherence, which under the effects of a gradient pulse will acquire a phase

$$G_p \xrightarrow{\gamma G_r r t} G_p\, e^{-ip\gamma G r t}$$

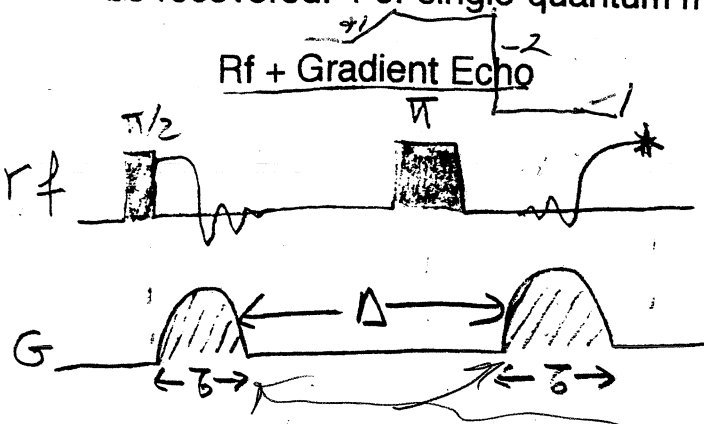
- $p = 0$ (zero-q coherences, populations) don't dephase
- $p = 2$ (double-q coherences) dephase twice as fast



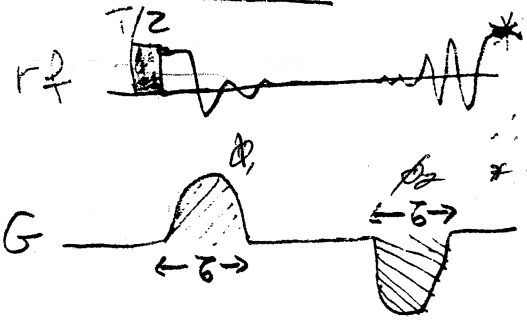
can unwind provide order of coh Doesn't change.

There are a number of ways by which signals dephased with gradients can be recovered. For single-quantum magnetizations:

Rf + Gradient Echo



Gradient Echo



Phases must $\therefore \phi_1 + \phi_2 = 0$ & cancelling phases

Δ will be diffusion-limited

Must be close together because molecules diffuse (slow DIFF in big molecules)

For an arbitrary change $p_1 \to p_2$ in coherence order the echo happens when

$$\phi_1 + \phi_2 = 0$$

eg) S.Q. → D.Q
eg) π pulse

where

$$\phi_1 = \gamma_1 G_z^1 z\, p_1 \tau_1$$

$$\phi_2 = \gamma_2 G_z^2 z\, p_2 \tau_2$$

τ_1, τ_2: lengths of pulses
G_z^1, G_z^2: pulse amplitude

$$\Rightarrow \quad \boxed{\frac{p_2}{p_1} = -\frac{\gamma_1 G_z^1 \tau_1}{\gamma_2 G_z^2 \tau_2}}$$

Coherence transfer pathways are selected according to the ratios between p's rather than according to their difference (as in phase cycling), but **in a single scan**.

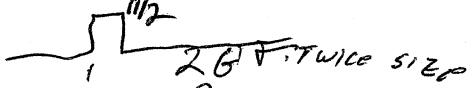
( ) + ( ) = 0 pathway changes

Watch out for coh

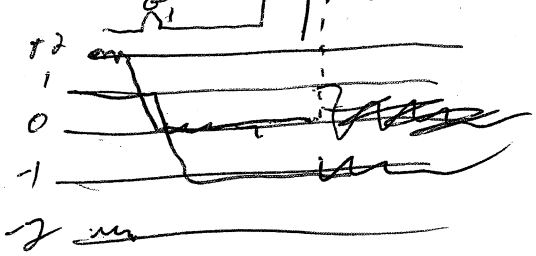
Disadvantages:

i) It is difficult to apply strong, fast gradient pulses due to eddy current effects (interaction with magnet, shims and metals)

ii) Gradient pulses can disturb the lock signal



The solution: self-shielding gradients and special lock hardware



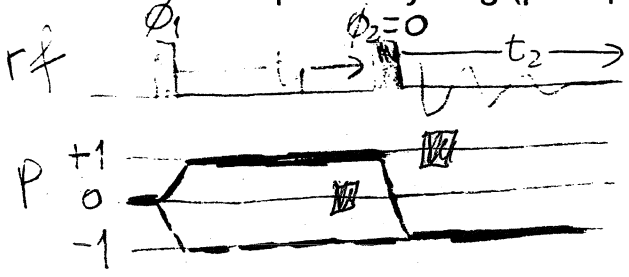
* WINDING ∴ MUST be DIFF
* Selective

eg) $\phi_1 = \gamma_H p \tau$

$\phi_2 = -2\gamma_H p \tau$

Example: 2D H,H-COSY NMR

1) Conventional phase cycling (pure phase w/quadrature in 6 scans)

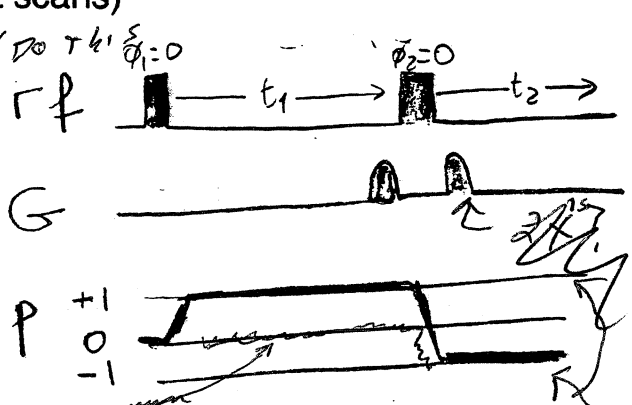


echo { $\phi_1 = 0, 120, 240$; $\phi_{Rx} = 0, 240, 120$

anti-echo { $\phi_1 = 0, 120, 240$; $\phi_{Rx} = 0, 120, 240$

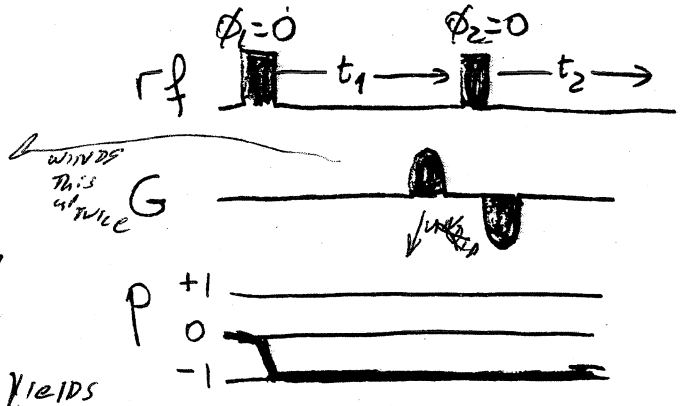
Gradient enhanced coherence selection (pure phase w/quadrature in 2 scans)

2) or Do This $\phi_1=0$ $\phi_2=0$



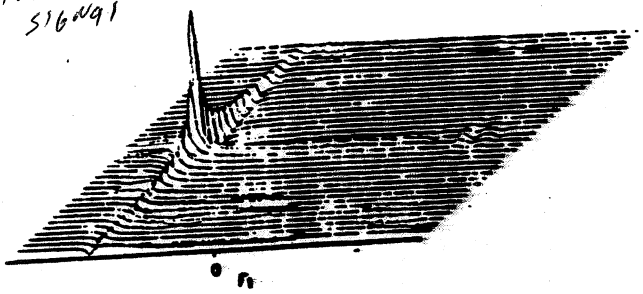
This would give no signal

only echo survives

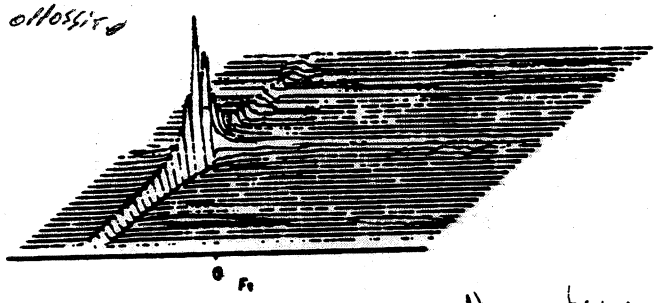


Yields opposite windings

only antiecho survives



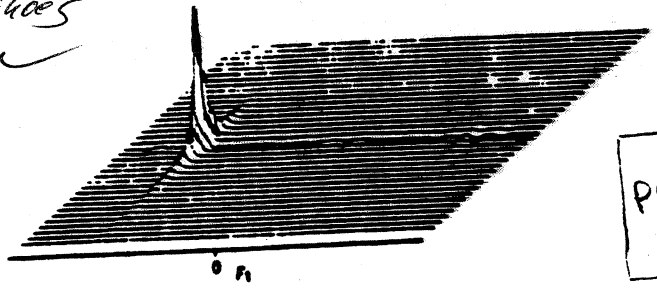
echo spectrum



antiecho (axis-reversed) spectrum

⊕

* Get echos & Antiechos in one scan



purely absorptive spectrum

VI.10 MULTIPLE-QUANTUM FILTERED COSY

The dispersive character of the COSY diagonal can produce

- cancellation among the diagonal peaks (because of change of sign)
  - diags dispersive
  - ↕ come from sine and cos mod
  - Additive cross peaks
- overlapping with the weaker cross-peaks (because of line width)

* crosspeaks, $I_{x_1} I_{z_2}$, must be antiphase?
* eliminate all single ops by Quantum Filtering
* Note diags have been single spin ops.
* we will Filter everything that isn't D.Q.C.

The origin of this problem lies in the fact that whereas the diagonal peaks arise from in-phase single-spin operators:

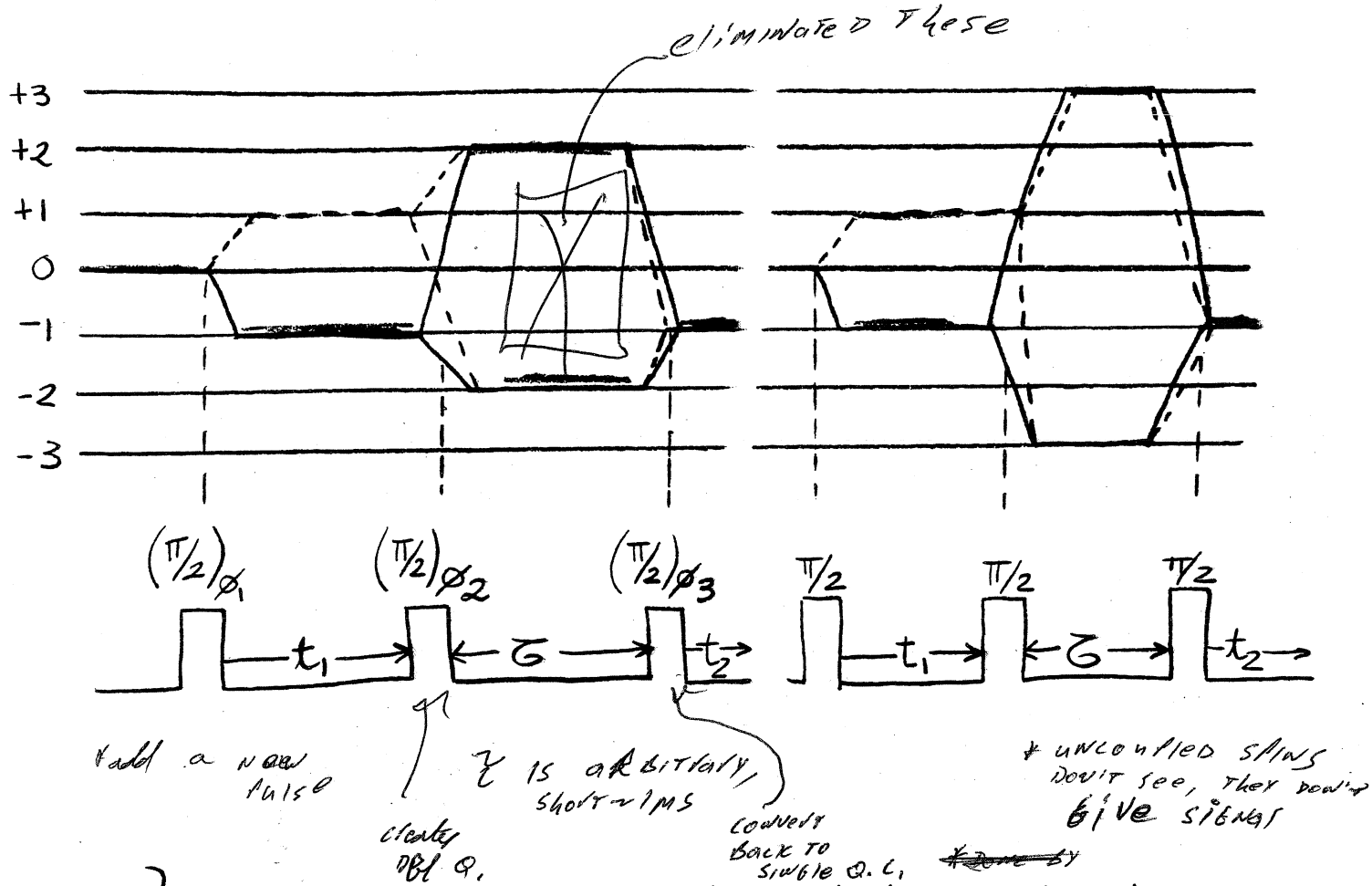
$$I_{z_1} \xrightarrow{(\pi/2)_y} I_{x_1} \xrightarrow{t_1} I_{y_1} \xrightarrow{(\pi/2)_y} I_{y_1} \xrightarrow{t_2} \text{diagonal peak}$$

cross-peaks arise from anti-phase two-spin operators

$$I_{z_1} \xrightarrow{(\pi/2)_y} I_{x_1} \xrightarrow{t_1} 2I_{x_1}I_{z_2} \xrightarrow{(\pi/2)_x} 2I_{z_1}I_{x_2} \xrightarrow{t_2} \text{cross peak}$$

The solution to avoid the problems caused by the diagonal is to filter out the single-spin operators (which don't give cross-peaks anyway) using a multiple-quantum filtration, i.e., **using a coherence pathway that requires the presence of coupling among at least a pair of spin operators.** The resulting pulse sequence should therefore have a coherence transfer pathway of the type:

Double-quantum filtered (DQF) H,H-COSY TQF H,H-COSY



The length of τ is very short, typically ≈ 10 μs; just enough to make a good rf phase shift.

Let's compare the transfer diagrams of conventional H,H- and of DQF-COSY experiments for a pair of coupled spins:

H,H-COSY

$$I_{z_1} \xrightarrow{(\pi/2)_y} I_{x_1} \xrightarrow[\text{Cs}]{t_1} I_{y_1} \xrightarrow{(\pi/2)_y} I_{y_1}$$: Diagonal peak.

(dispersive diag peak)

$$\downarrow$$

$$2I_{x_1}I_{z_2} \xrightarrow{(\pi/2)_y} 2I_{z_1}I_{x_2}$$: cross peak.

Triple q. coh $= \left(\frac{1}{2}\right)^{n-1} = 1/4$ lost

DQF-COSY

ignore single spin ops...

$$I_{z_1} \xrightarrow{(\pi/2)_y} I_{x_1} \xrightarrow{t_1} 2I_{y_1}I_{z_2}$$ ← sin mod

$$\downarrow (\pi/2)_y$$

$$-2I_{y_1}I_{x_2} = \underbrace{-\frac{1}{2}i\left(I_{1-}I_{2+} - I_{1+}I_{2-}\right)}_{\text{ZQC (Phase-cycled out)}} \underbrace{-\frac{i}{2}\left(I_{1+}I_{2+} - I_{1-}I_{2-}\right)}_{\text{DQC (kept)}}$$

(ZQC cycled out for gradient)

$= 1/2$ of signal lost! ∴ new pulse disadv, need 4x's as long S/N

Does it matter for 1/1 does for 1/2

$$\rightarrow \frac{i}{2}\left(I_{1+}I_{2+} - I_{1-}I_{2-}\right) = \frac{1}{2}\left(2I_{x_1}I_{y_2} + 2I_{y_1}I_{x_2}\right)$$

$$\downarrow \quad (\pi/2)_x \quad \downarrow$$

$$-\frac{1}{2}\left(2I_{x_1}I_{z_2} + 2I_{z_1}I_{x_2}\right)$$

$2I_{x_1}I_{z_2} \rightarrow I_{y_1}$ diagonal peak

$2I_{z_1}I_{x_2} \rightarrow I_{y_2}$ cross peak

resonates a c.s. of spin 1

Both are sine modulate in t_1; both phasecible

Note that in a DQF-COSY both diagonal- and cross-peaks come from anti-phase two-spin coherences; therefore, they are both absorptive and give antiphase doublets with respect to the active coupling (as always, there are in-phase splitting due to the passive couplings).

Note also that there is a 1/2 factor involved, and therefore the transfer is only 50% as effective as that of a normal COSY. This loss of sensitivity however, is more than compensated by the fact that the diagonal is no longer dispersive:

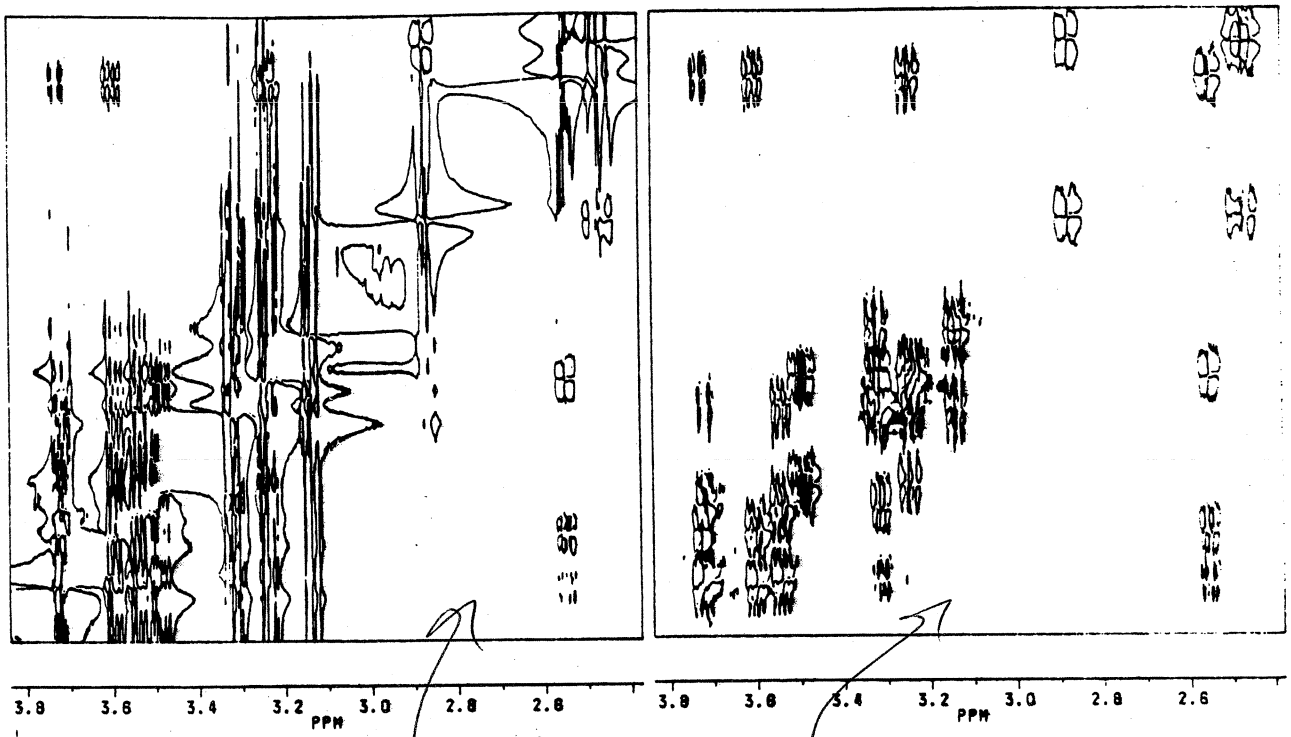


Figure 8.37 A comparison of normal phase-sensitive COSY (left, same data as Figure 8.27) with DQF-COSY (right). The tremendous clarification of the diagonal region of the latter spectrum more than compensates for the theoretical loss in sensitivity in this experiment.

regular COSY

DQF COSY
* those broad risers along diag which are dist.
* water signal gone, can't see D, Q, C's.

DQF-COSY is one of the most useful techniques for determining J-couplings even when they are very small. It can be used together with the Karplus equation to get information about molecular conformations.

Double-quantum filtering is implemented by phase cycling. There are 3 pulses => in principle there are $4^3 = 64$ possible combinations.

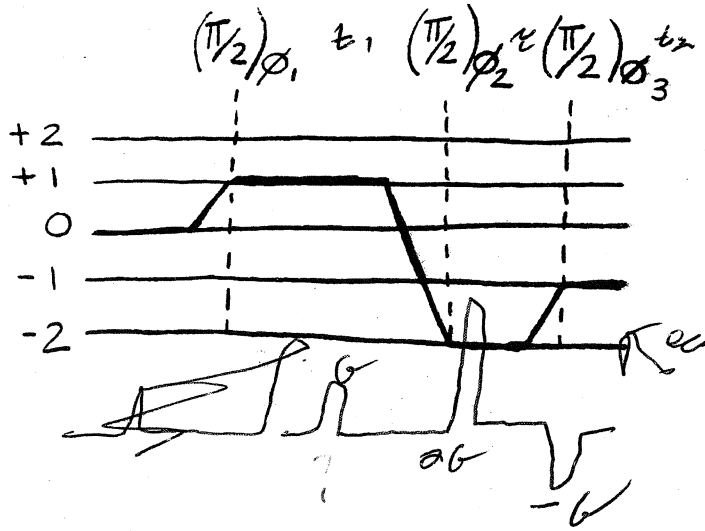
The first 16 (i.e., with $\phi_3 = 0$)

ϕ_1	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
ϕ_2	0	0	0	0	1	1	1	1	2	2	2	2	3	3	3	3
ϕ_3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ϕ_{Rx}	0	3	2	1	3	2	1	0	2	2	0	3	1	0	3	2

Quad, no ghosts — get quad ghosts in t_1 but t_2 out — $\Rightarrow$ t_2 Quad

$\phi_3 = 0000\ 1111\ 2222 \ldots$ 64 terms — ghosts now

The receiver phase is determined by the coherence transfer pathway



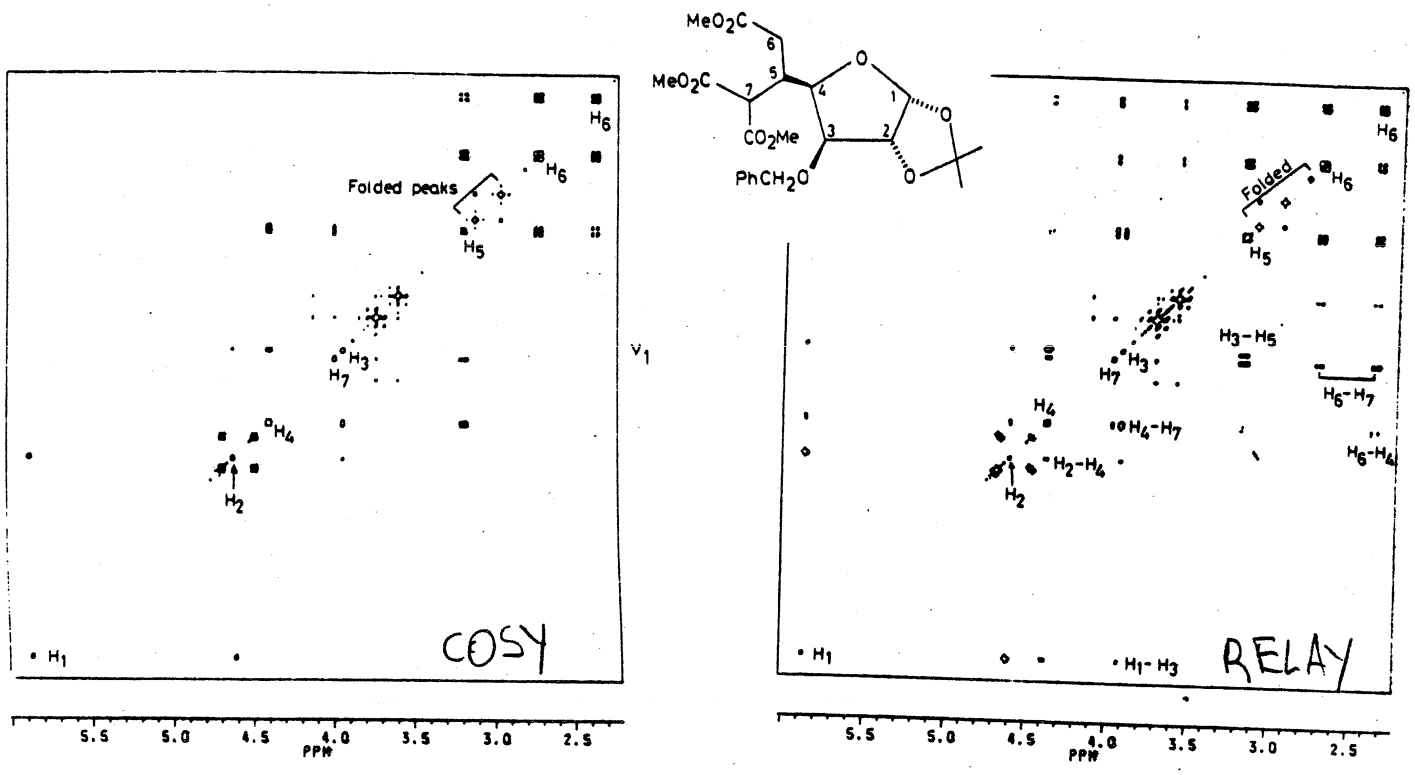
$$\phi_{Rx} = -\{(+1)\cdot\phi_1 + (-3)\cdot\phi_2 + (+1)\cdot\phi_3\}$$

set R_x like this

$$\phi_{Rx} = -\phi_1 + 3\phi_2 - \phi_3$$

For the 9th column for instance, $\phi_{Rx} = 2$

Actually, both normal COSY as well as **relayed** (i.e., indirectly coupled) cross-peaks are observed in the final spectrum:



The efficiency of the transfer is proportional to

Transfer Func $\Rightarrow \sin(\pi J_{12}\Delta)\cdot\sin(\pi J_{23}\Delta)$;

DIAGNOSTIC OF TYPES OF RESIDUES

Δ per. depts effic.

and therefore an a priori knowledge of the couplings helps to set up the experiment * works w/ H's, C=O Terminate coup eq)

* ^{13}C enriched, N will ??

A completely different strategy is used during a **TOCSY** (**TO**tal **C**orrelation **S**pectroscop**Y**) experiment for obtaining cross-peaks among inderectly coupled sites. The ideal mixing-period Hamiltonian of a TOCSY NMR experiment is:

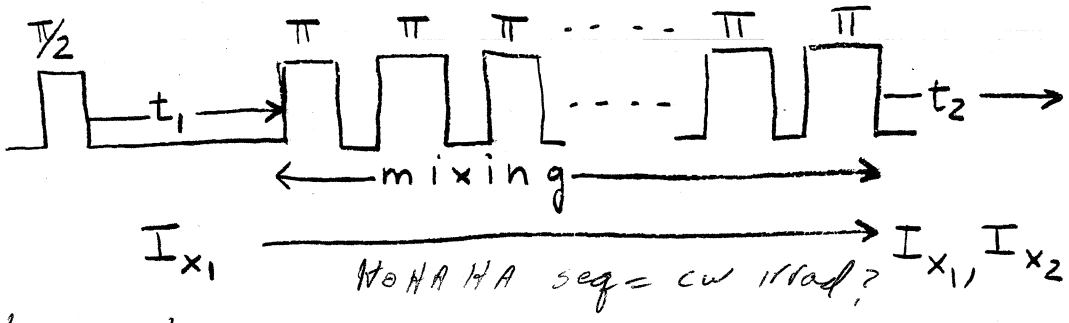
* TOCSY → ~~STRONGLY~~ WEAKLY coupled AX AX SYSTEMS

* TOCSY ELIMINATES CS. DIFF.; GET A A' A'' A''' S...

$$\mathcal{H}^{(m)} = \sum_{k<l} J_{kl}\, \vec{I}_k \cdot \vec{I}_l$$

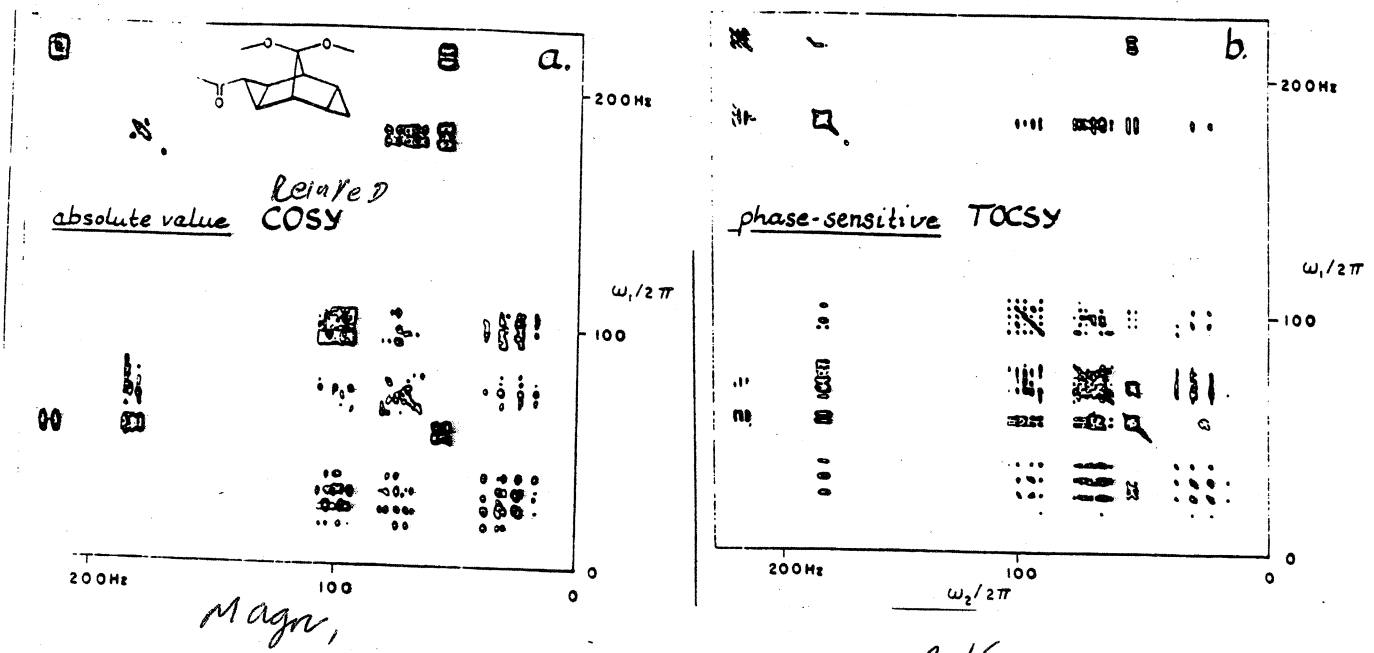
scalar Hcd,

All chemical shifts are gone, thus transforming all systems of coupled spins into the type AA'A"...; a simple (although not the optimum) way of minimizing the effects of the chemical shift evolution is using a train of π pulses



NO C.S. & ~~zero~~ J coupling, SPIN LOCK ANALOGY ∴ get equal INTENS QE ~~600~~ C.P.'s

Since the transfer involves amplitude modulations, purely-absorptive line shapes can be obtained. During the mixing there are "modes" of coherence transfer, the resulting spectra are similar to those of relayed-COSY:

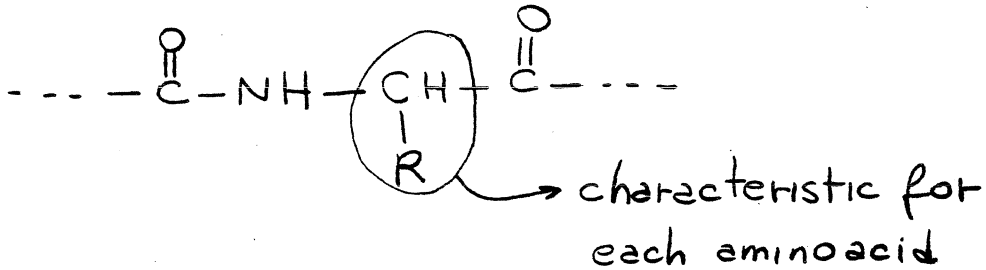


Magn,

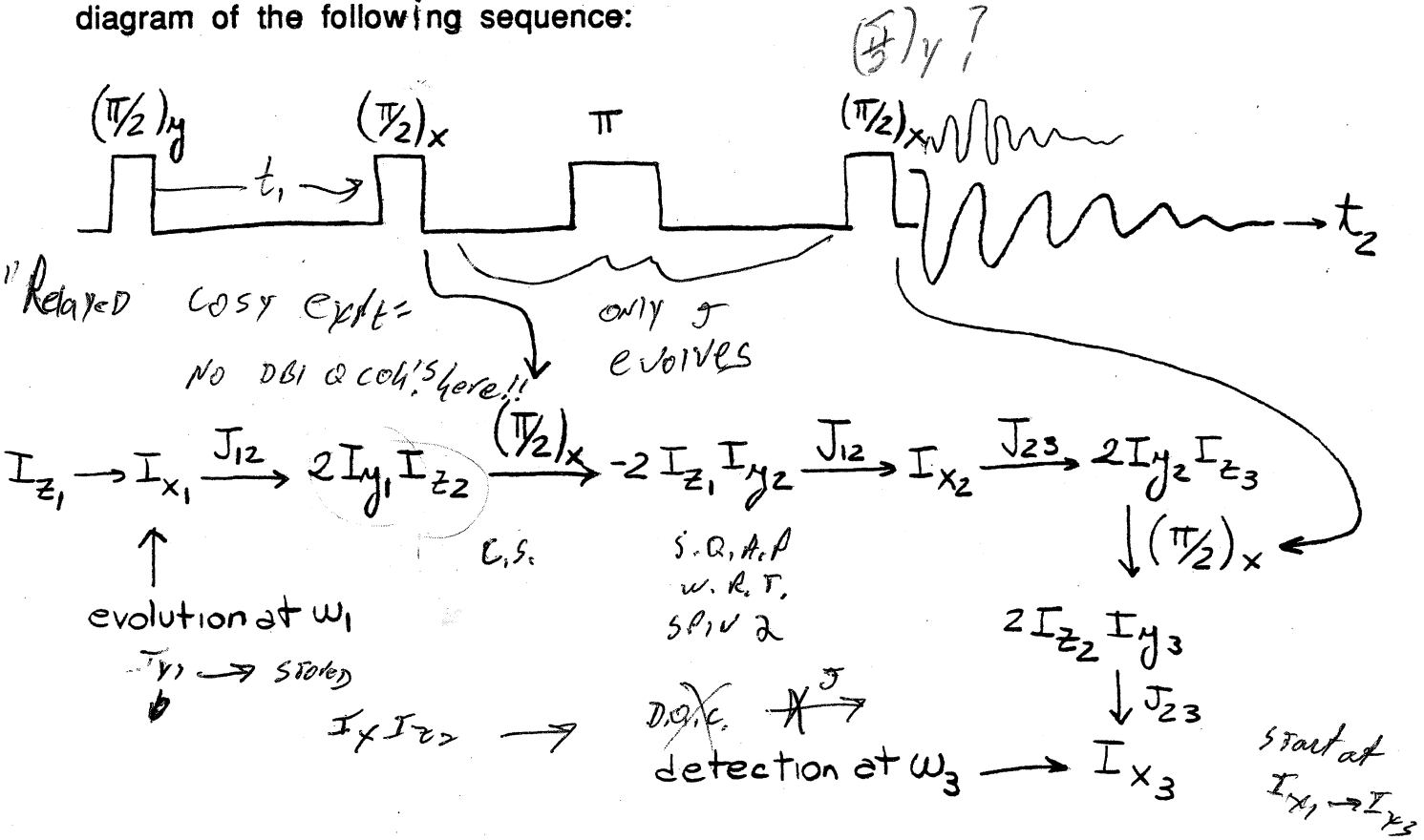
All give C.P.'s, w/equal INTENSITY

VI.11 CROSS PEAKS AMONG INDIRECTLY COUPLED SPINS: RELAYED COSY AND TOCSY NMR EXPERIMENTS

So far we have only considered experiments where coherence is transfered among pairs of directly coupled spins. These experiments however do not identify **systems** of coupled spins, a valuable information when trying to assign for instance the origin of resonances in peptides:

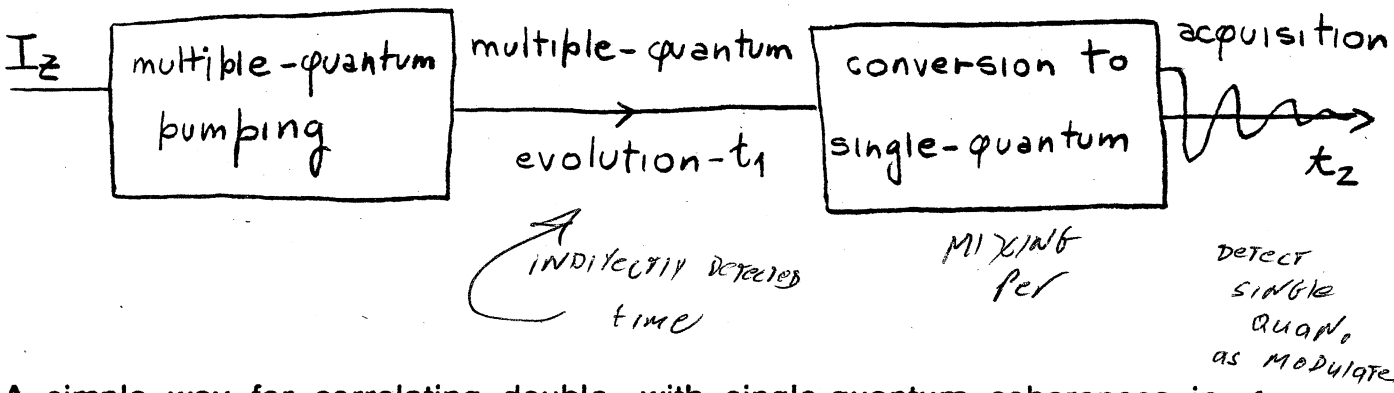


Suppose that we have a three-spin system {1, 2, 3}, with $J_{12} \approx J_{23} \neq 0$; $J_{13} = 0$. A COSY spectrum yields cross peaks between 1 and 2, and between 2 and 3; but doesn't give cross-peaks between spins 1 and 3. Consider however the transfer diagram of the following sequence:

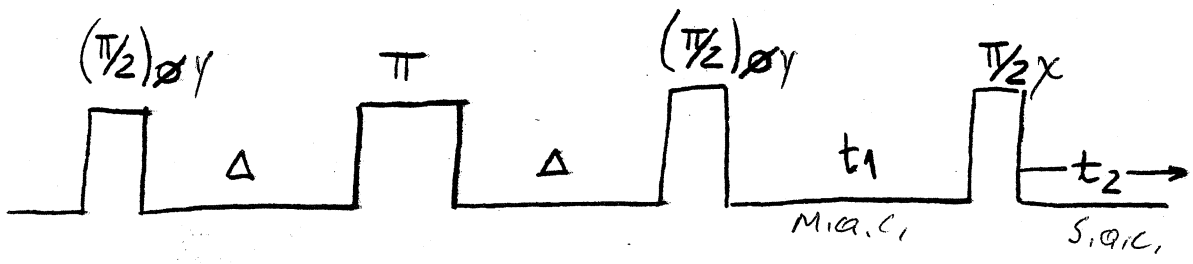


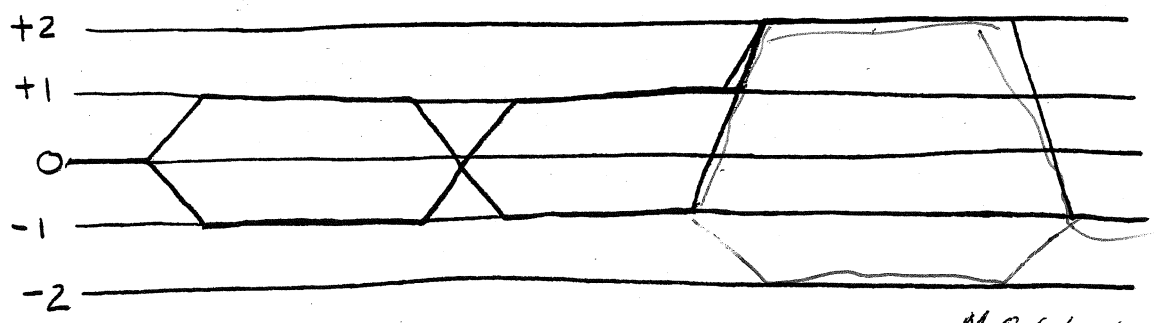
VI.11 MULTIPLE-QUANTUM SPECTROSCOPY: THE INADEQUATE EXPERIMENT

We saw how multiple-quantum evolution can be used as a "filter" to simplify multi-D NMR spectra. Multiple-quantum spectroscopy attempts direct observation of the multiple-quantum coherences, by correlating them with the single-quantum coherences that originate from them. The scheme of multiple-quantum spectroscopy:



A simple way for correlating double- with single-quantum coherences in homonuclear systems is by using the **INADEQUATE** (Incredible Natural Abundance DoubIE QUAntum Transfer Experiment) sequence:





M.Q. COH NEED'D) 2 SPINS

MONITORING M.Q. COH AS FUNC OF t_1

2 OR (π/2) 2 PULSES

The π-pulse in the pumping allows us to disregard the effects of chemical shifts. The transfer diagram for a pair of spins then becomes

symmetry

$$I_{z_1} + I_{z_2} \xrightarrow{(\pi/2)_y} I_{x_1} + I_{x_2} \xrightarrow{\Delta-\pi-\Delta} 2I_{y_1}I_{z_2} + 2I_{z_1}I_{y_2}$$

$$\xrightarrow{(\pi/2)_y} 2\left(I_{x_1}I_{y_2} + I_{y_1}I_{x_2}\right)$$

D.Q.C. & Q.Q.C.

$$\downarrow (\omega_1 I_{z_1} + \omega_2 I_{z_2})t_1$$

(there's no coupling evolution)

only C.S. evolution

$$2\left(I_{x_1}I_{y_2} + I_{y_1}I_{x_2}\right)\cos\left[(\omega_1+\omega_2)t_1\right] + 2\left(I_{y_1}I_{y_2} - I_{x_1}I_{x_2}\right)\sin\left[(\omega_1+\omega_2)t_1\right]$$

becomes unobservable

lose the D.Q.C's

$$\downarrow (\pi/2)_x$$

non-quad in t_1

Get back to phase cycle

$$2\left(I_{x_1}I_{z_2} + I_{z_1}I_{x_2}\right)\cos\left[(\omega_1+\omega_2)t_1\right]$$

C.S. of 1 evolve @ t_2

C.S. of 2 evolves

$$\downarrow (\omega_1 I_{z_1} + \omega_2 I_{z_2} + J I_{z_1}I_{z_2})t_2$$

$$I_{x_1}\cos\left[(\omega_1+\omega_2)t_1\right]\sin(\omega_1 t_2)\sin\left(J\frac{t_2}{2}\right) + I_{x_2}\cos\left[(\omega_1+\omega_2)t_1\right]\sin(\omega_2 t_2)\sin\left(J\frac{t_2}{2}\right)$$

* Each have own C.S. & split by J → a.p. doublets

eg) $^{13}C - ^{12}C - ^{12}C - ^{13}C - ^{13}C$

* only ^{13}C–^{13}C pairs → D.Q. coh's → detectable signal *

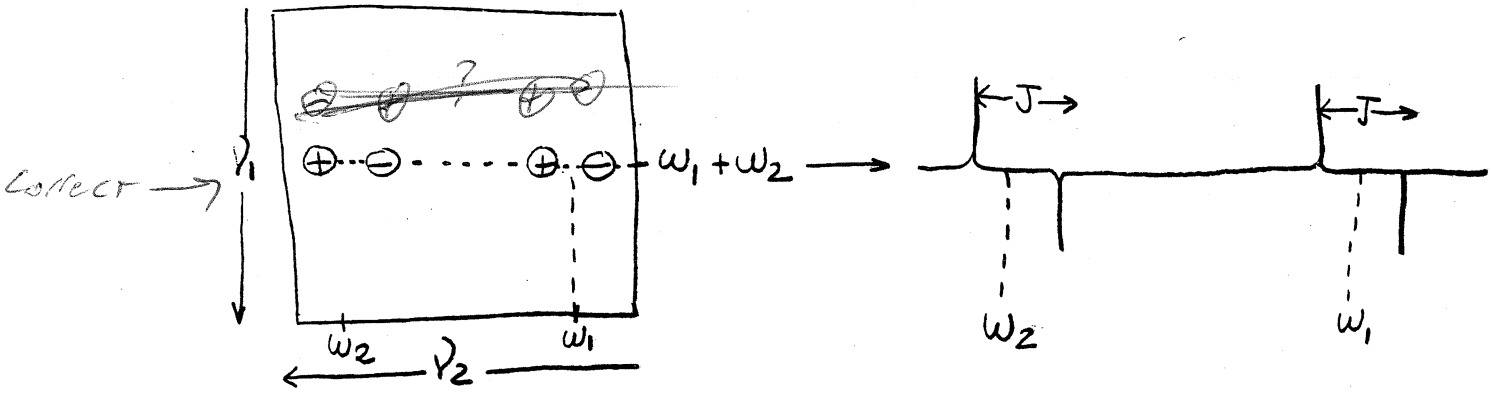
eg) $CH_3\,CH_2\,CH_2\,CH_2OH$

1	2	3	4
13	13	12	12
12	13	13	12
12	12	13	12

only worry about this

over

There are no splittings along the double-quantum dimension; pairs of antiphase doublets are observed along the single quantum dimension:



This experiment is usually used to extract molecular connectivities from natural abundance ^{13}C NMR spectra: the probability of finding a ^{13}C-^{13}C pair is $\sim \frac{1}{10{,}000}$, small but observable. The spectral analysis: $\frac{1}{100} \times \frac{1}{100} = \frac{1}{10000}$

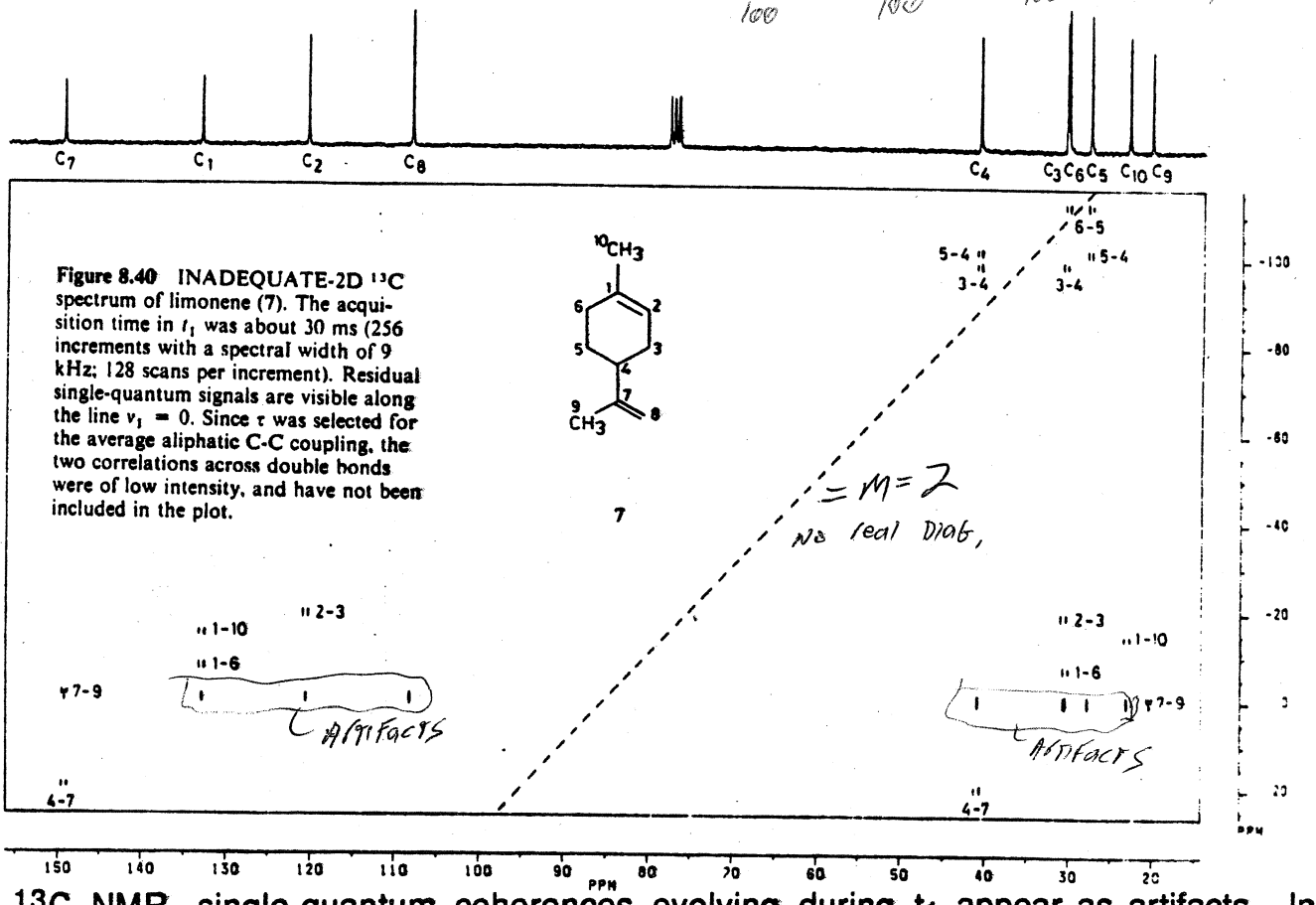


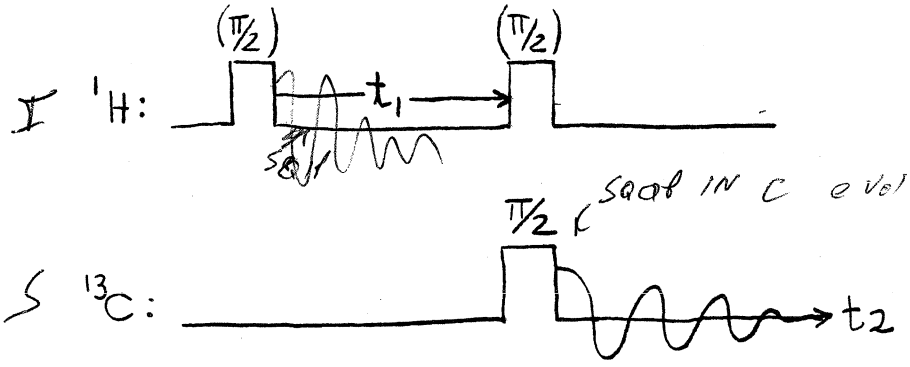
Figure 8.40 INADEQUATE-2D ^{13}C spectrum of limonene (7). The acquisition time in t_1 was about 30 ms (256 increments with a spectral width of 9 kHz; 128 scans per increment). Residual single-quantum signals are visible along the line $\nu_1 = 0$. Since τ was selected for the average aliphatic C-C coupling, the two correlations across double bonds were of low intensity, and have not been included in the plot.

In ^{13}C NMR, single-quantum coherences evolving during t_1 appear as artifacts. In ^1H NMR there are usually more than 2 coupled spins in a system, and the spectrum becomes very complex.

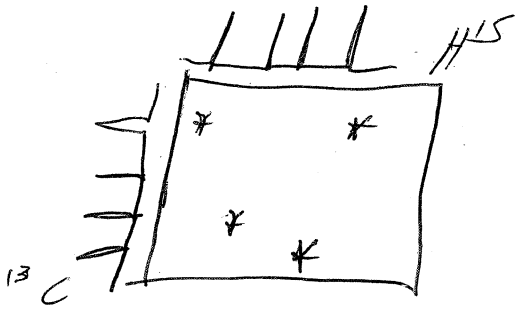
Artifacts, come from those cycl, gradients work better though

VI.13 2D HETERONUCLEAR CORRELATION SPECTROSCOPY

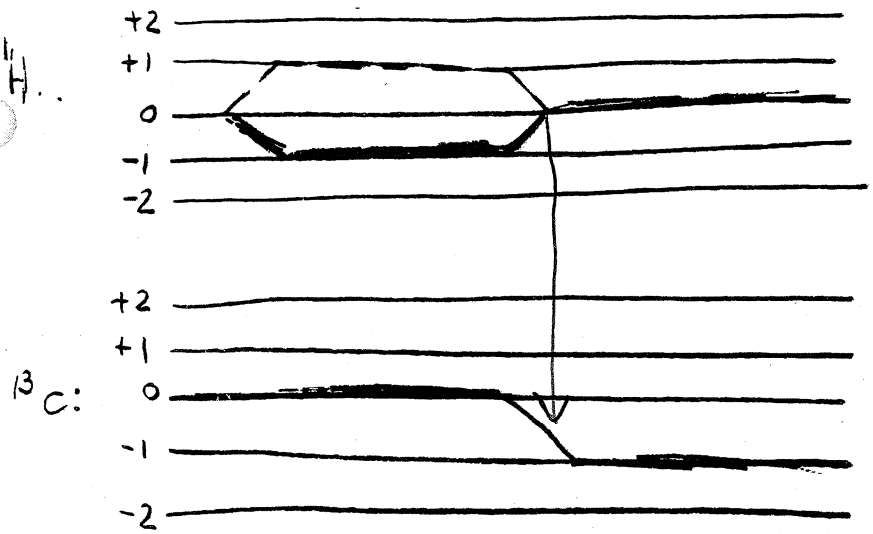
The first pulse sequence that we analyzed when investigating heteronuclear coherence transfer, can be transformed into a 2D NMR experiment useful for elucidating which ^{1}H is bonded to which ^{13}C in a molecule:



This sequence is equivalent to the first H,H-COSY sequence that we saw



one peak each? hmwk

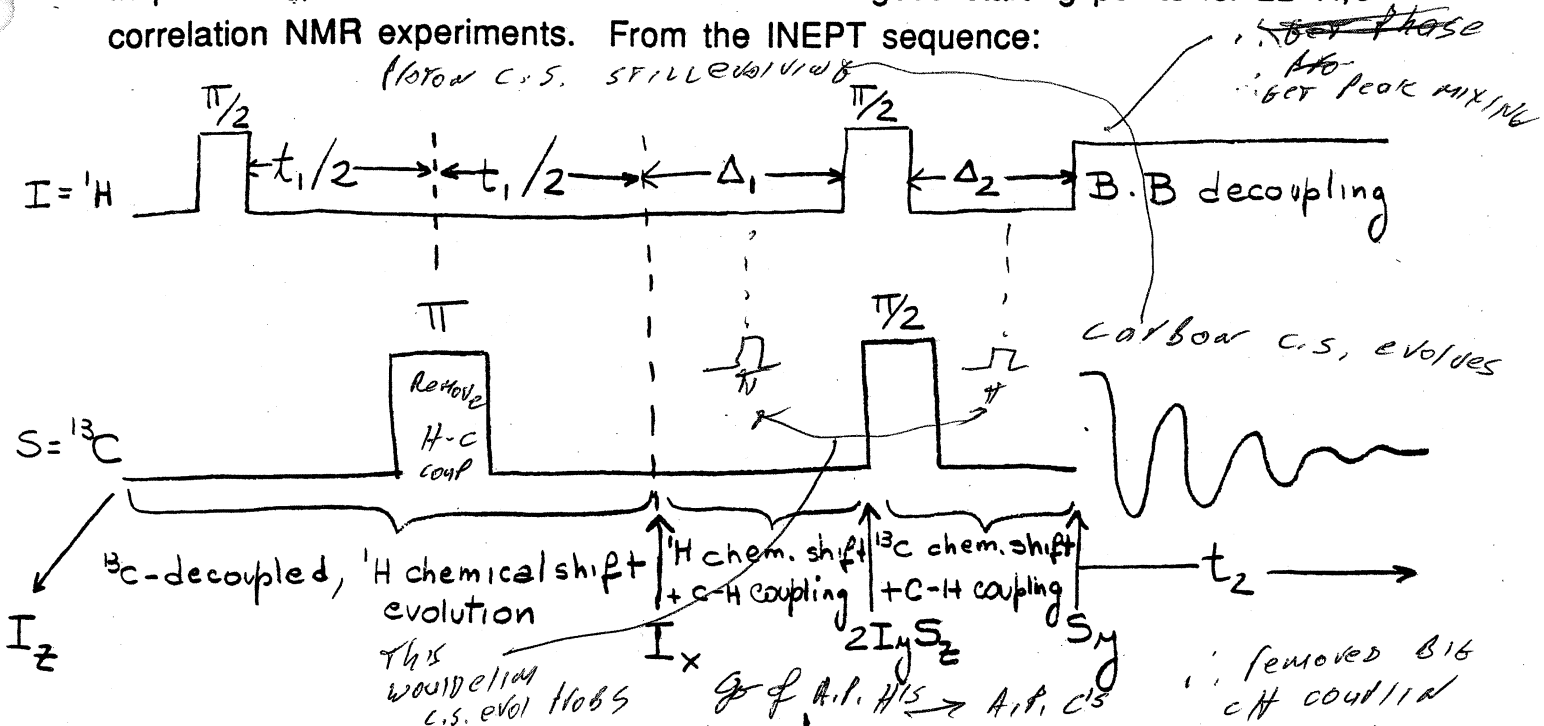


important step

*MUST consider whole ON of H's & ¹³'s
* have probs of J-coupl...
* FINAL Hof.

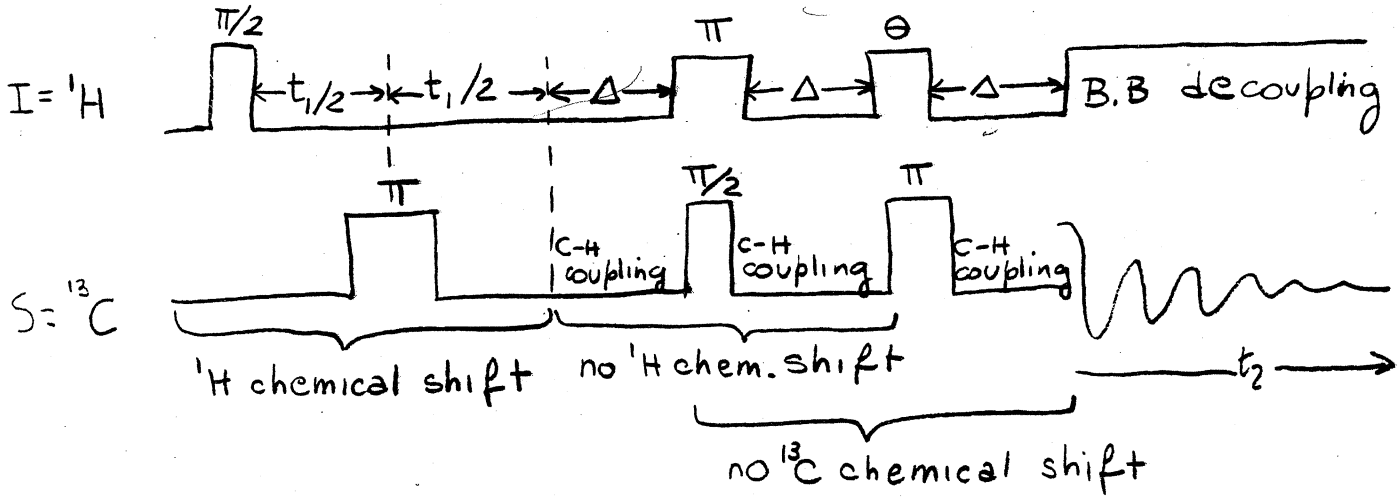
Of course, much better schemes can be implemented for obtaining coupling-free spectra and purely-absorptive line shapes.

In particular, both the INEPT and DEPT make good starting points for 2D H,C-correlation NMR experiments. From the INEPT sequence:



This sequence has the drawback that the ^{1}H chemical shifts evolved during Δ_1 => it's difficult to get purely-absorptive phased spectra.

A better choice is the sequence coming from DEPT:



No chemical shift evolution is present for $t_1 = t_2 = 0$!

2D heteronuclear COSY's are extremely powerful tools for spectral assignment, structural elucidation, and for enhancing the spectral resolution of ^1H NMR.

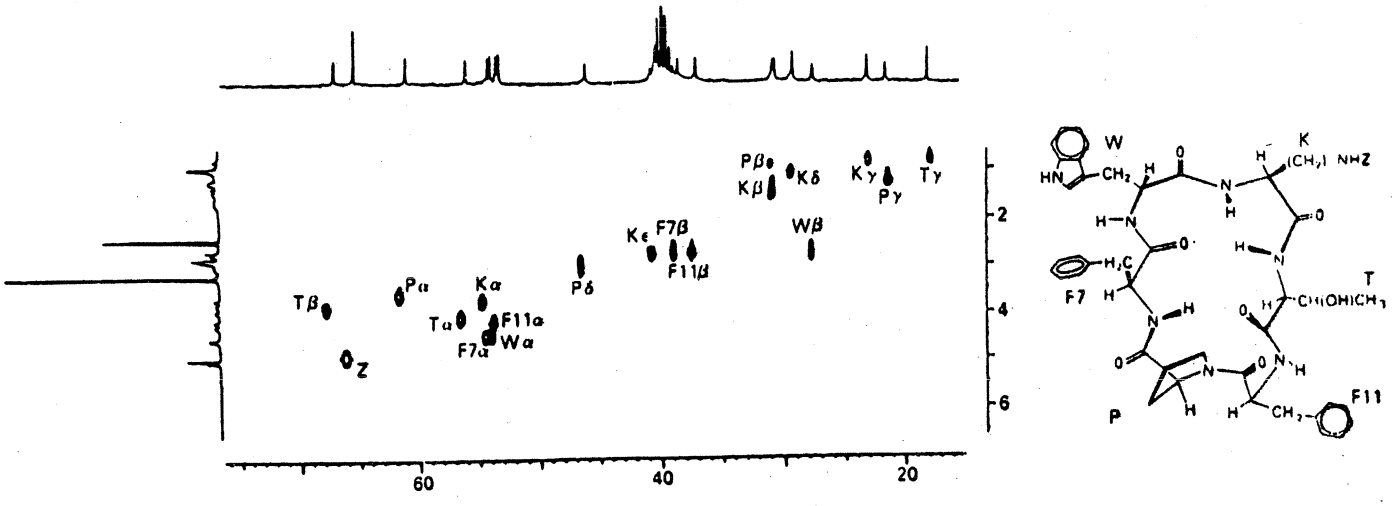
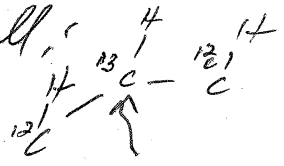


FIG. 8.5.4. Molecular structure of the hexapeptide *cyclo* [Phe^7-D-Trp-Lys(Z)-Thr-Phe^{11}-Pro] and heteronuclear proton–carbon shift correlation spectrum, obtained with the sequence of Fig. 8.5.3(c). The 1D proton spectrum is shown on the left, and the proton-decoupled carbon-13 spectrum on top (aliphatic region only). Note the peak-shapes that appear elongated in the vertical ω_1-dimension because of ill-resolved homonuclear proton–proton couplings. Many signals that are well-resolved in the 2D spectrum overlap in either of the 1D spectra. (Reproduced from Ref. 8.88.)

* IN 2D these projections no longer overlap

* hmwk remove; H-H & H-C couplings

* recall, 

can distinguish theses, ~~decay~~ only rotation &
Decouple w.r.t, w/ BB pulses ..!

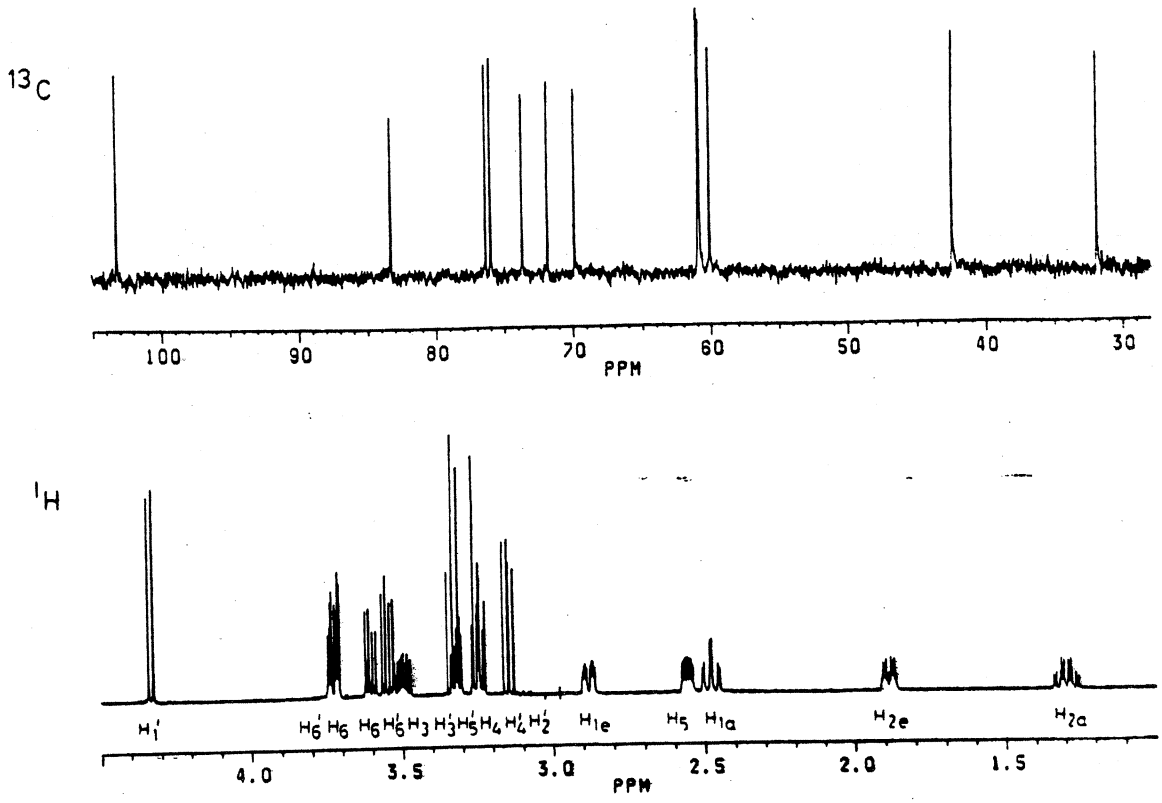
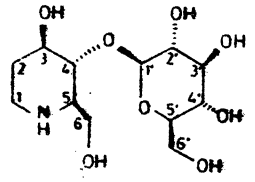


Figure 9.4 Proton and carbon spectra of compound 1. The carbon spectrum was acquired using DEPT (400 scans). on the same sample used for the HSC experiment in the following figure.



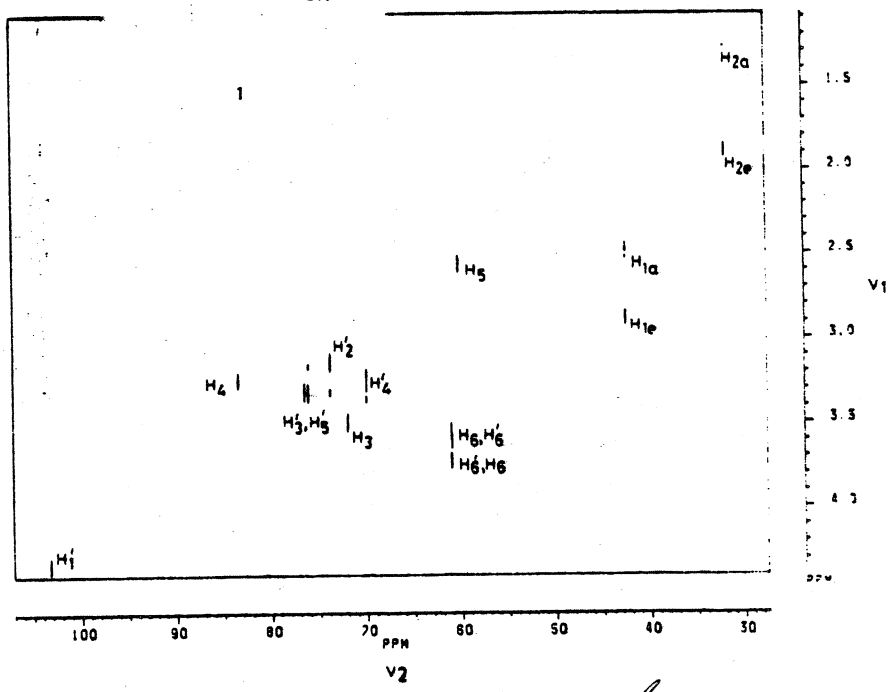


Figure 9.5 HSC spectrum of 1 (128 t_1 increments for an acquisition time in that dimension of 76 ms; 192 scans per increment). The proton assignments (derived using COSY in the previous chapter) are marked. In the crowded central region it is a little difficult to identify relative proton shifts from the contour plot, but these were readily measured by examining vertical slices through the spectrum. The assignments of H_4 and H_4' may be interchanged.

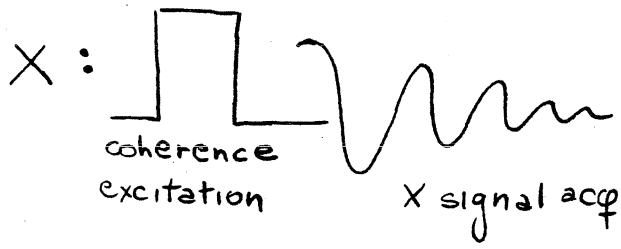
'H & C spectrum

* See 2H's Due To Rate of Inversion?

VI.14 INVERSE SPECTROSCOPY

Most hot Hetcod exft

Consider a heteronuclear spin pair H-X, where one of the spins is a proton and X = ^{13}C or ^{15}N. To observe the time-domain NMR signal of X, we started with the simplest experiment:

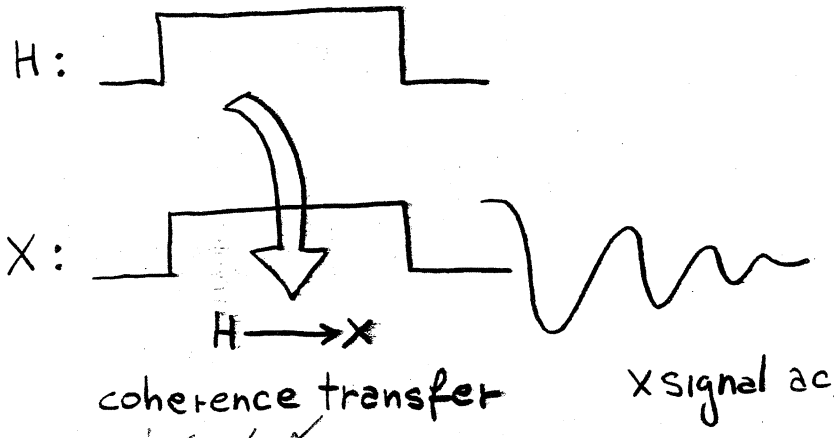


direct excitation
direct detection

signal : $S_{d,d}$

and got a signal $S_{d,d}$ from which the spectrum could be obtained. Then, by making an INEPT-type sequence, we saw that in terms of S/N at least a factor γ_H / γ_X could be gained due to the fact that the equilibrium magnetization of ^1H is larger than the one from the X spins:

Recall INEPT



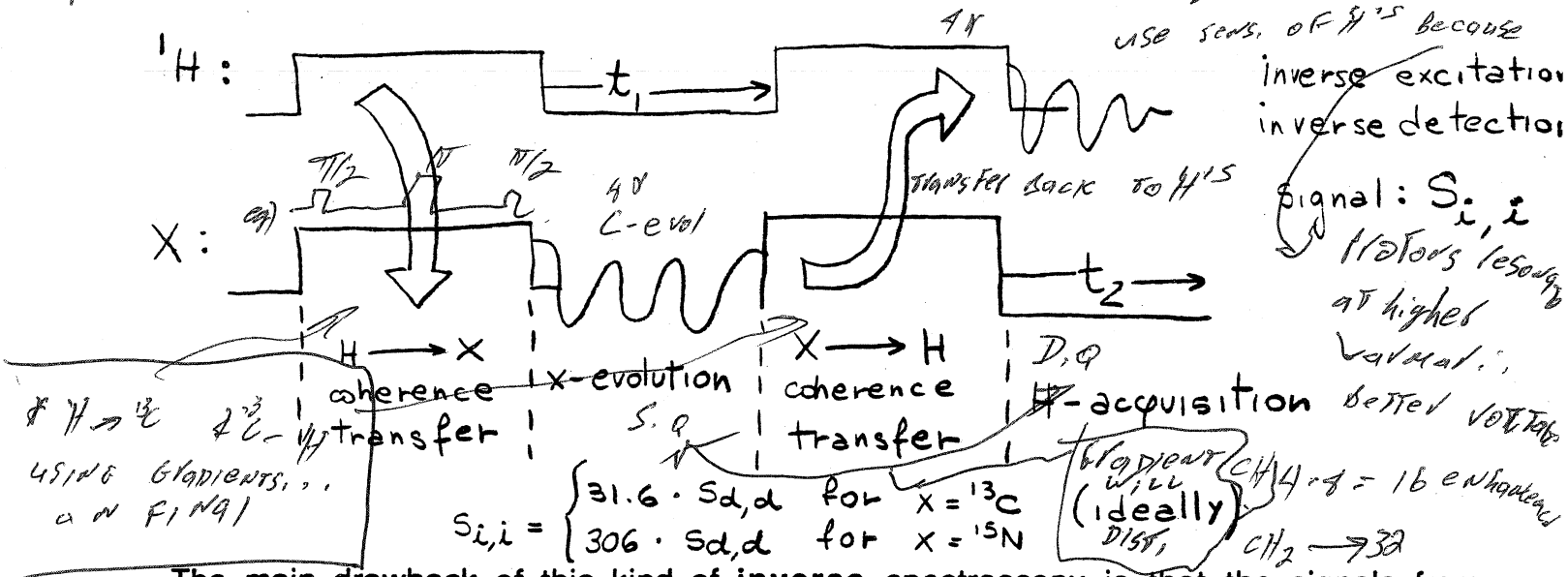
inverse excitation
direct detection

signal : $S_{i,d}$

∴ Gain $\frac{\gamma_H}{\gamma_X}$

$$S_{i,d} = \begin{cases} 4 \cdot S_{d,d} & \text{for } X = {}^{13}C \\ 9.9 \cdot S_{d,d} & \text{for } X = {}^{15}N \end{cases}$$

Even further sensitivity enhancements can be achieved if the X-spin evolution is transfered back to the protons and detected at the ^1H NMR frequency:

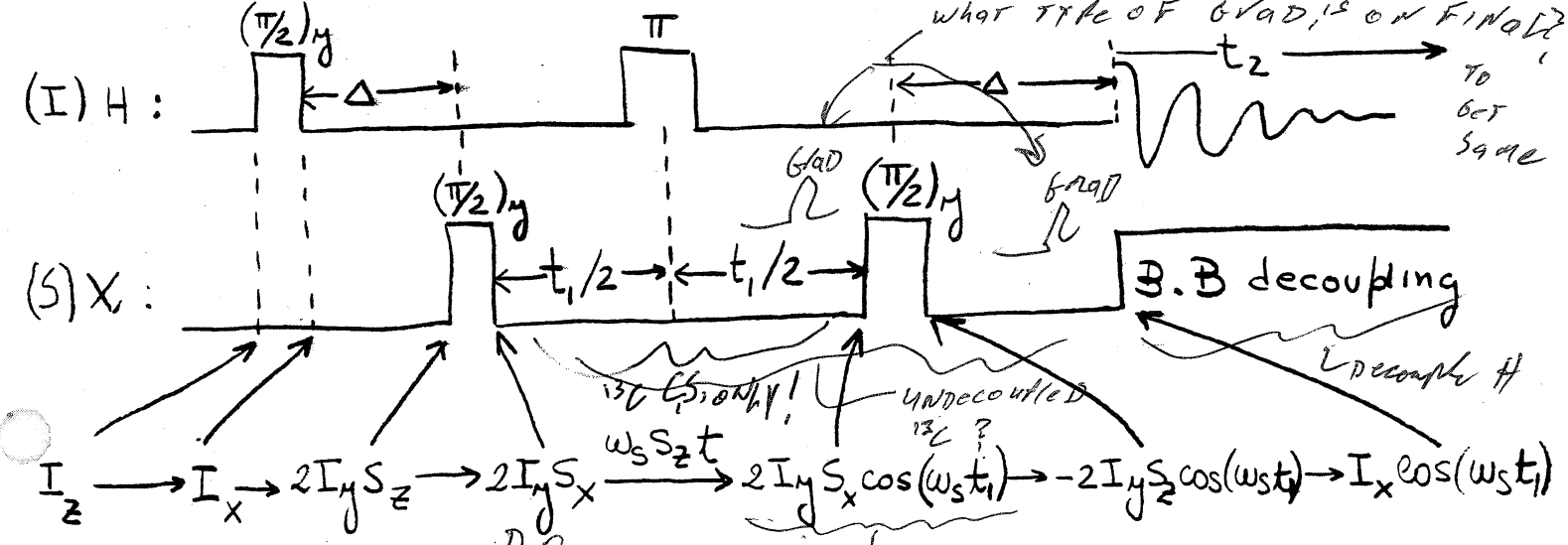


The main drawback of this kind of **inverse** spectroscopy is that the signals from the overwhelming majority of the protons (which are not coupled to any dilute X spin) have to be suppressed very efficiently. This problem can be alleviated by two means:

i) Inserting a BIRD-type pulse to excite only protons bonded to active X spins.

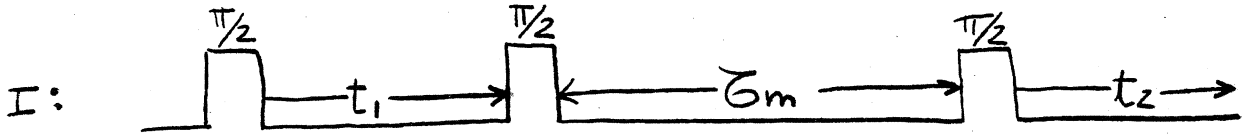
ii) Using **multiple-quantum** H-X coherences for monitoring the chemical shift evolution of X during t_1. If no active X spins are present, no ^1H signal during t_2 should result.

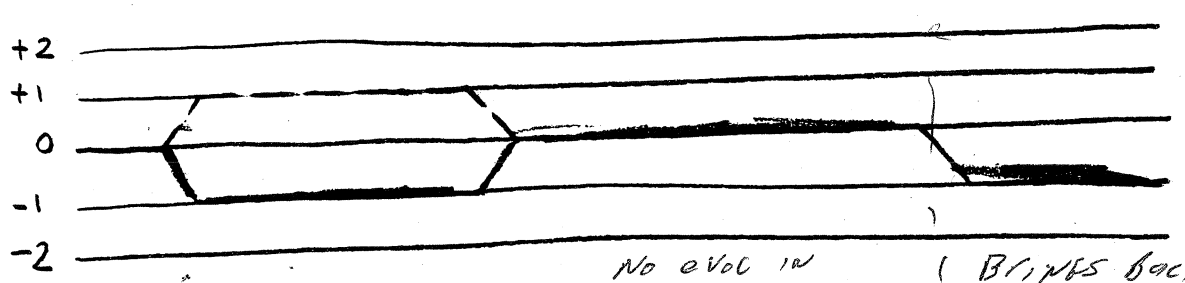
A simple way of implementing these type of inverse experiments is using the H-detected Heteronuclear Multiple-Quantum Coherence (the HMQC) pulse sequence



VI.15 EXPERIMENTS INVOLVING TRANSFER OF Z-MAGNETIZATION; 2D EXCHANGE NMR

Let's consider again the pulse sequence that we used for the DQF-COSY experiment, but this time focussing on a different coherence transfer pathway:



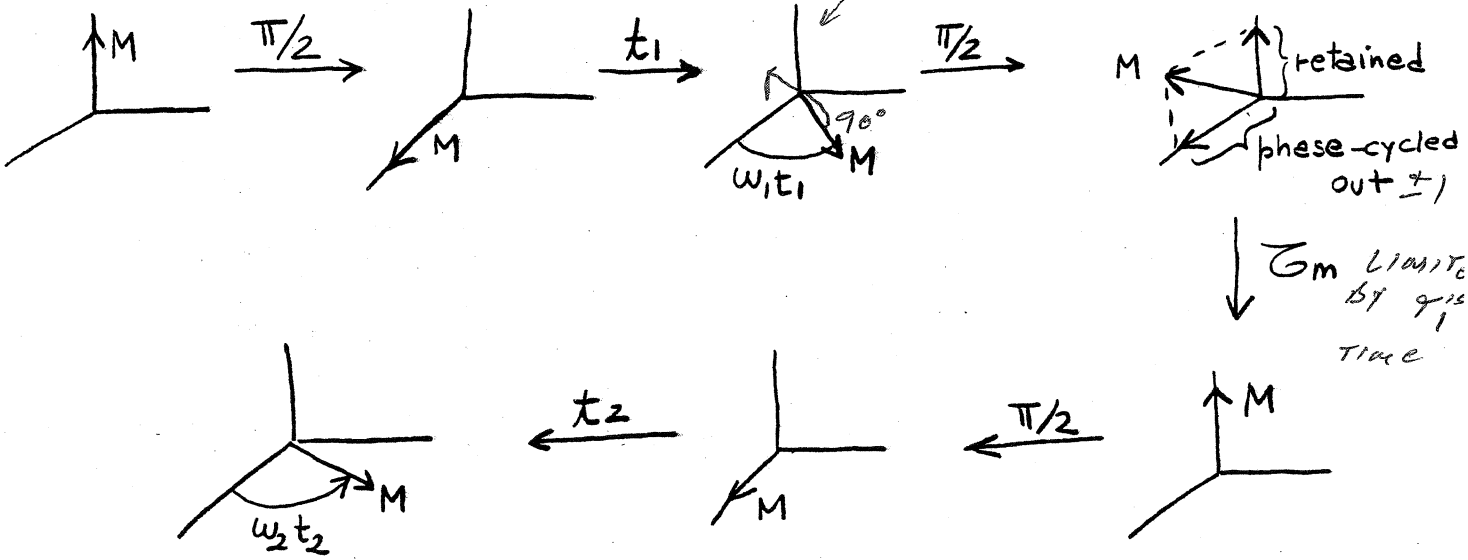


±1 & 0 ∴ can talk in Vectors

No evol in mixing ∴ store magnet along z (Brings back

If we have a system composed by uncoupled spins, this is one of the few 2D NMR sequences that can be understood using the classical magnetization model. Indeed, since in these systems the zero-coherence pathway is actually equivalent to z-magnetization, then

Not at equil ∴ see mag C_1's (Later Pg's)



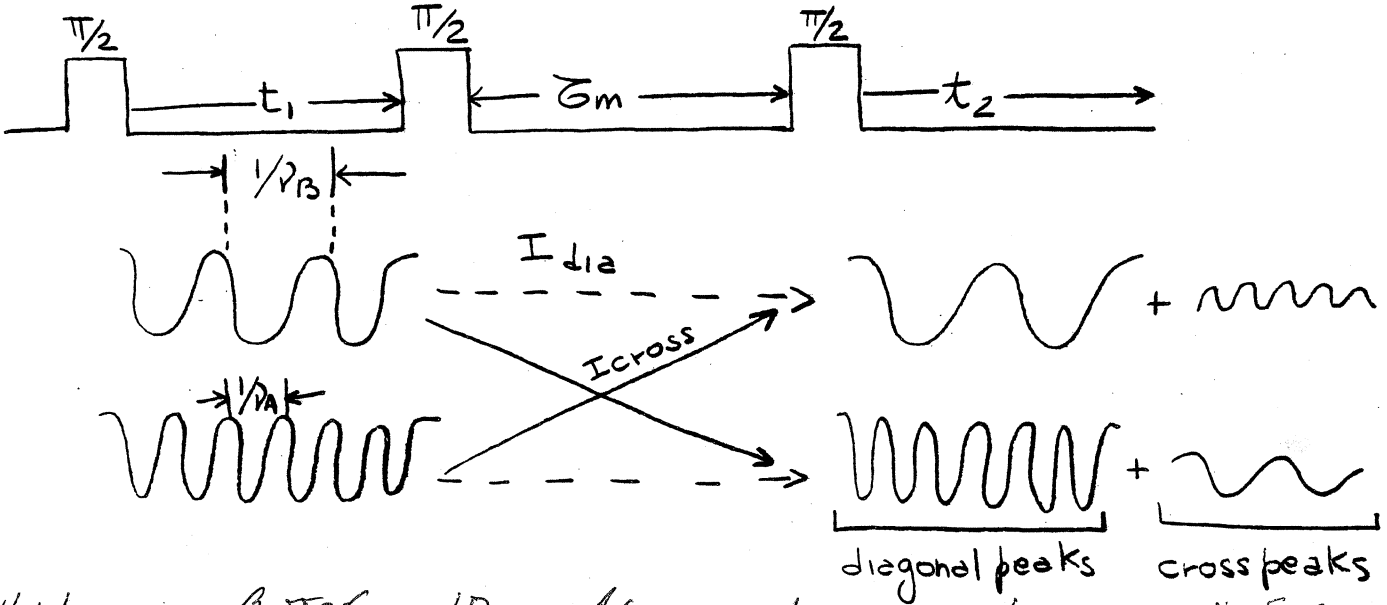
eg) CH_3 before & after mixing & if have chem exch ovlp

& T_1 long compared to W_{rec}, & correct freq at $t_1 - W_1$ & $t_2 - W_2$

If the precession frequency of the spins does not change during the mixing time τ_m, one gets a 2D NMR spectrum where peaks appear at frequencies $\omega_1 = \omega_2$ (i.e., along the diagonal). The precession frequency can change however if one has a site undergoing an exchange process in the slow-exchange regime:

site A $\underset{}{\overset{k}{\rightleftharpoons}}$ site B $\qquad k \ll |\nu_A - \nu_B|$

Then, during the mixing period (which can be in the order of 0.1-1.0 sec):



* which more better, 1D expt much better & easier, allows select excit

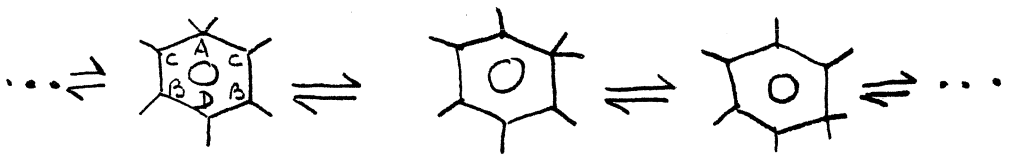
* use this for excn of several sites, good for sev sites

The 2D NMR spectrum obtained by $\mathcal{F}$T-ing the signal $S(t_1,t_2)$, **gives a map of the sites coupled by the chemical exchange process.** Moreover, in simple cases (equally populated sites, linear approximation for the exchange, etc.), one can get an estimate of the exchange rate from the ratio

KIN. 1ST order Analysis

$$\frac{I_{dia}}{I_{cross}} \simeq \frac{1 - k \cdot \tau_m}{k \cdot \tau_m}$$

An example of how this information can become available from the 2D spectra appears in the behavior of the heptamethylbenzonium ion



1,2 methyl shift taking place in 9.4 M H_2SO_4

* slow on NMR time scale

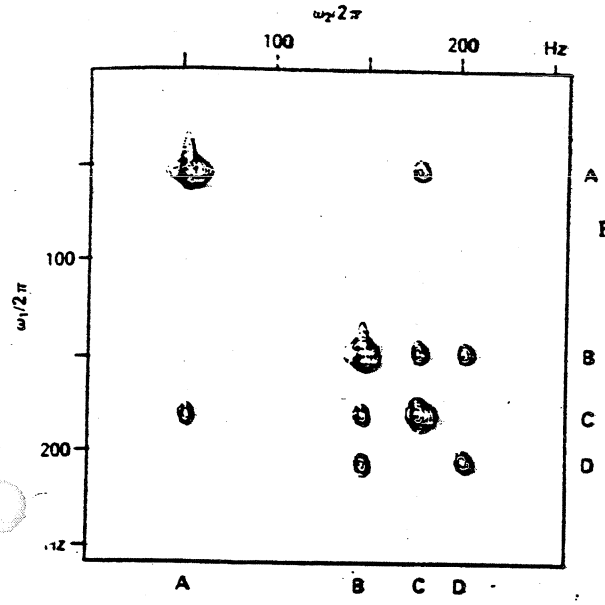


FIG. 9.8.2. Two-dimensional exchange spectrum of the protons in heptamethylbenzenonium ion in 9.4 M H_2SO_4 obtained with the sequence in Fig. 9.1.1(a) with τ_m = 280 ms. The cross-peak amplitudes are consistent with a 1–2-alkide shift mechanism. (Reproduced from Ref. 9.2.)

* could not analyze w/ INV exch.

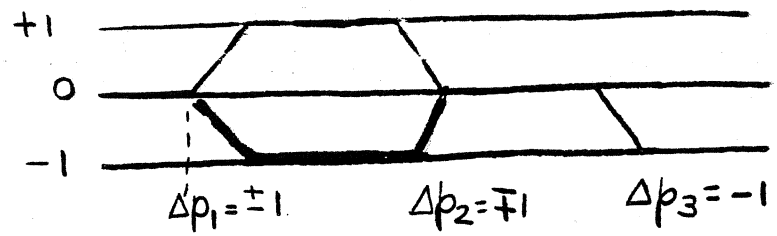
The phase cycling required for this 2D exchange NMR spectroscopy sequence depends on what kind of system we have and on what kind of line shapes we want to observe

i) If we have no spin couplings

a $\Delta p = 2$ mask (i.e., phase-cycling involving rf phase shifts of 180°) is enough $\{0, \pi\}$

ii) If we want purely-absorptive line shapes, we have to keep both ±1 coherence pathways during t_1

only have ±1 cohs i, easy cycling



$$\phi_{R_x} = \mp \Delta\phi_1 \pm \Delta\phi_2 + \Delta\phi_3$$

A good approach is to keep the phase of ϕ_3 constant, so that magnetization which doesn't come from $\Delta p = \pm 1$ during t_1 cancels out

$\Rightarrow$

$\Delta\phi_1$	0	2	0	2
$\Delta\phi_2$	0	0	2	2
$\Delta\phi_3$	0	0	0	0
ϕ_{Rx}	0	2	2	0

← keep const, because T_m is in order of relax.; signal which relaxes has no t_1 mod.) ; cancel large peaks (axial)

iii) If in addition one wants quadrature detection in t_1, the +1, -1 coherence pathways during t_1 have to be collected independently.

VI.16 NOESY AND 2D ROESY NMR EXPERIMENTS

Most remarkably, it was found that when the 2D exchange NMR pulse sequence also was applied to molecules in which no chemical exchange processes were taking place, **cross-peaks among proximate sites could still be observed!**

cross relax. effect (NOE)

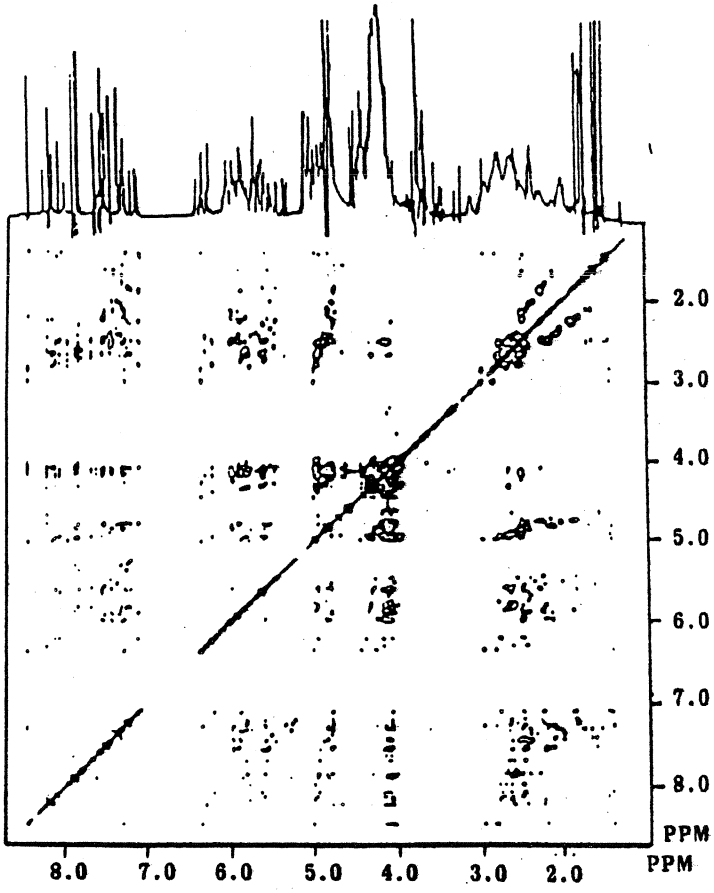


Fig. 9.3: NOESY spectrum of a DNA oligomer [illegible]

Cross-peaks at a frequency (ω_A,ω_B) in this 2D NMR experiment must have come from magnetization of site A which was precessing at a frequency ω_A during t_1, was transferred to site B during the mixing time, and started to precess at a rate ω_B after the 3rd pulse. **This transfer occurrs via cross-relaxation,** the same phenomenon which originated the NOE. Indeed, recall that the longitudinal relaxation of two proximate homonuclear spins I,S was given by:

R_S : self-relaxation R_C : cross-relaxation

$$\frac{dS_z}{dt} = -\overbrace{(W_0 + 2W_1 + W_2)}(S_z - S_z^\circ) - \overbrace{(W_2 - W_0)}(I_z - I_z^\circ)$$

S → equil I → equil

If Not at equil ⇒ have $I \xrightarrow{\text{rate}} S$ & $S \to I$

$$\frac{dI_z}{dt} = -(W_2 - W_0)(S_z - S_z^\circ) - (W_0 + 2W_1 + W_2)(I_z - I_z^\circ)$$

1) Noe → cross peaks (H's must be close though)

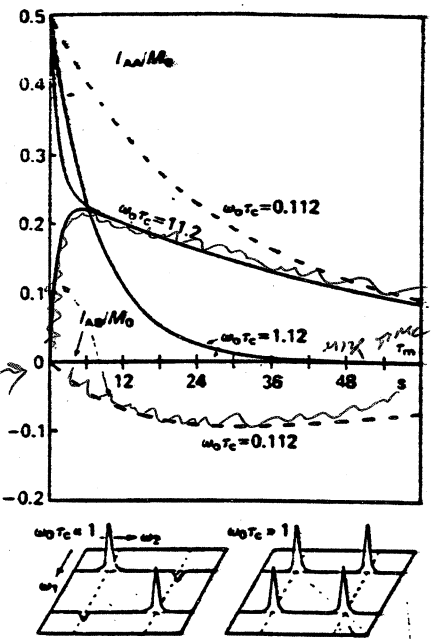
2) chemexch → " " # all for same exp DT !

Since the magnetization brought into the z-axis during the mixing is not in thermal equilibrium, it will relax partly into S and partly into I magnetization. This will originate a cross peak between the chemical shifts of sites I and S. The ratio between the diagonal- and the cross-peak intensity is a function of τ_m, of the spatial distance between the two sites, and of the correlation time τ_c of the vector connecting the sites which in turn controls the value of the relaxation rates W_i's. A plot of this latter dependence:

Quantification of Cr. PKS,

τ_m short or very long → small Cr. PK

$\frac{W_1}{?}$? $\frac{1}{\tau_c}$ Det size of Cr. PKS



= Cr. PKS

Black = Diagonals

Conversely can lose Cr PKS for longer τ_m's

* Note inverted PKS Due to eqn's

* For very very large τ_m's diags & Cr. PKS same intens, But usually broadened Due to short T_2's

FIG. 9.7.2. Dependence of the diagonal and cross-peak intensities $I_{AA} = I_{BB}$ and $I_{AB} = I_{BA}$ on the mixing time τ_m for cross-relaxation in an AB spin system. Three typical correlation times τ_c have been assumed: $\omega_0\tau_c = 0.112$ corresponds to a short correlation time (extreme narrowing, negative cross-peaks), while $\omega_0\tau_c = 11.2$ represents a case of long correlation time (slow motion, positive cross-peaks). The critical case $\omega_0\tau_c = 1.12$ leads to vanishing cross-peaks irrespective of the mixing time τ_m. The indicated time-scale assumes a Larmor frequency $\omega_0/2\pi = 100$ MHz and $q = 3.33 \times 10^9\ s^{-2}$. (Reproduced from Ref. 9.5.)

a) Mix → Yield

This type of **NOE** SectroscopY (**NOESY**) is very useful for determining tertiary structure of bio-macromolecules, as cross peaks depend on **spatial proximity** and not on the number of intervening bonds:

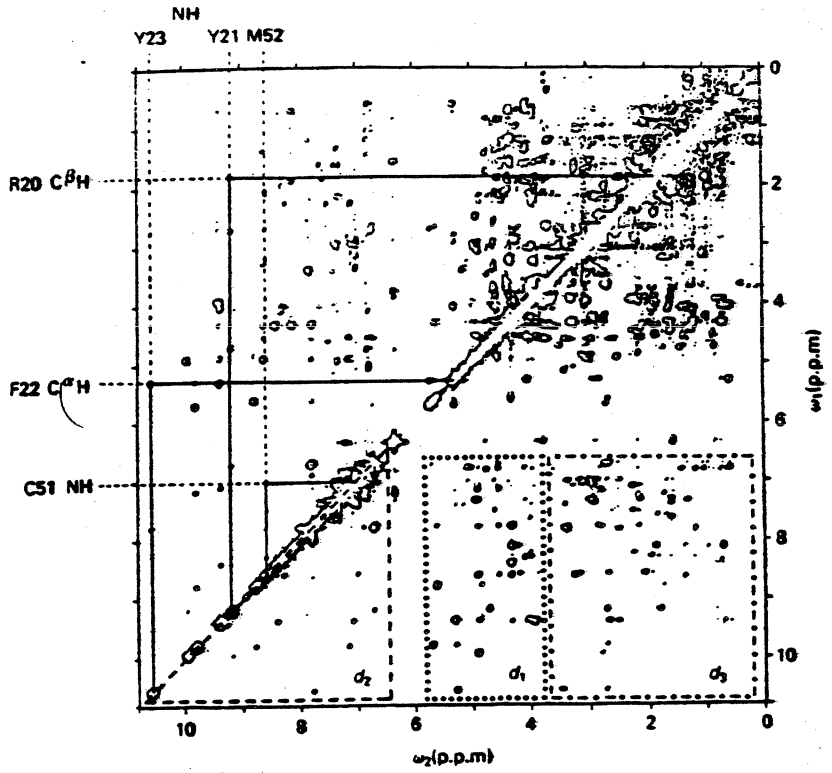


FIG. 9.7.4. Contour plot of a symmetrized, absolute-value 500-MHz ^{1}H NOESY spectrum of a 0.02 M solution of basic pancreatic trypsin inhibitor (BPTI) in D_2O, p*H 4.6, T = 36°C. The spectrum was recorded in ~6 h, immediately after dissolving the protein in D_2O, so that, in addition to the non-labile protons, the resonances of ~30 backbone amide protons are seen between 7 and 10.6 p.p.m. In the lower right triangle, three spectral regions of interest for sequential resonance assignments are outlined, i.e. the regions where NOE connectivities between different amide protons (---), between amide protons and C^{α} protons (. . . .) and between amide protons and C^{β} protons (- - - - -) are usually observed. In the upper left triangle, the assignment of one of each of these types of connectivity is shown (C = cysteine, F = phenylalanine, M = methionine, R = arginine, Y = tyrosine). (From Ref. 9.30.)

Folding of coiled protein yields many Cr. NOEs from NOE

know even w/out NOE still get cr. PKS.
Problem, can't quantify NOE PKS

This technique however, has two important drawbacks:
J-couPs, contrib thru D.Q. Coh's

i) In coupled spin systems zero quantum coherences originate cross-peaks which, although not related to relaxation effects, are very similar from the ones arising from the NOE:

survives mixing

$$I_z \xrightarrow{(\pi/2)_x} I_y \xrightarrow{J I_z S_z t_1} 2I_x S_z \xrightarrow{(\pi/2)_x} 2I_x S_y : \underset{+}{DQC} \underset{ZQC}{} \xrightarrow{\tau_m} (I_x S_y + I_y S_x)\cos[(\omega_z - \omega_s)$$

$$\xrightarrow{(\pi/2)_x} S_y \xleftarrow{J I_z S_z t_2} 2I_z S_x$$

Ho D.Q. Coh's last about T_2

They can be distinguished from the NOESY peaks by their τ_m dependence:

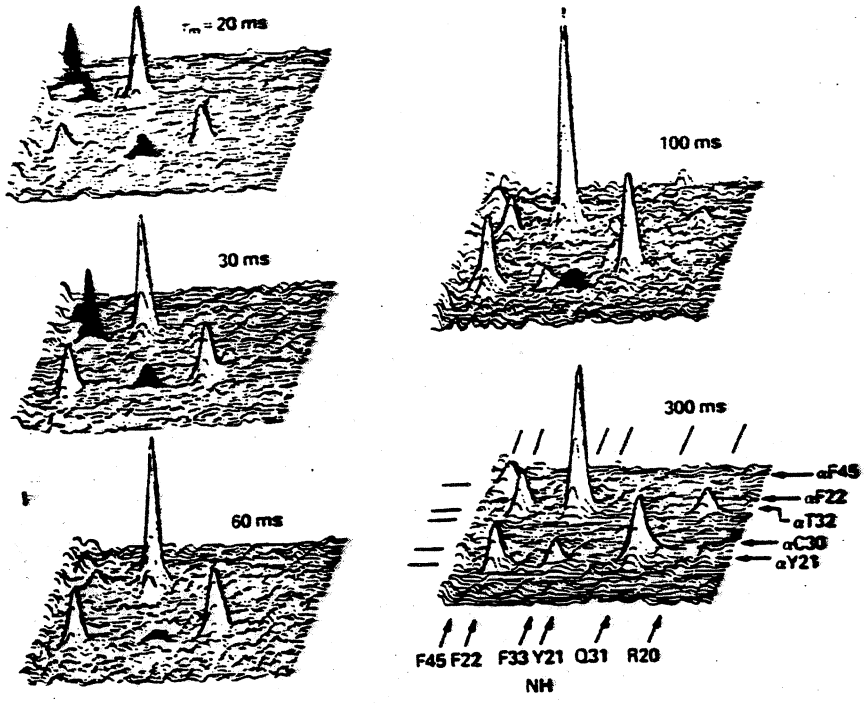
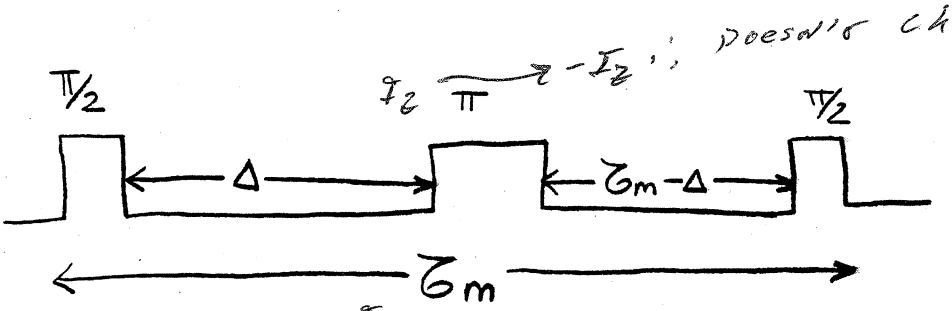


FIG. 9.7.5. Two-dimensional NOE spectra of basic pancreatic trypsin inhibitor (BPTI) for five different mixing times τ_m. A blow-up of the region $5 \leq \omega_1 \leq 6$ p.p.m. and $8 \leq \omega_2 \leq 10$ p.p.m. is shown. Abbreviations: C = cysteine, F = phenylalanine, Q = glutamine, R = arginine, T = threonine, Y = tyrosine. The blacks peaks are due to zero-quantum coherence (so-called '*J*-peaks', discussed in § 9.4.2). (Adapted from Ref. 9.15.)

Moreover if a π pulse is inserted at a time Δ during the mixing time and this period is allowed to change randomly from scan to scan



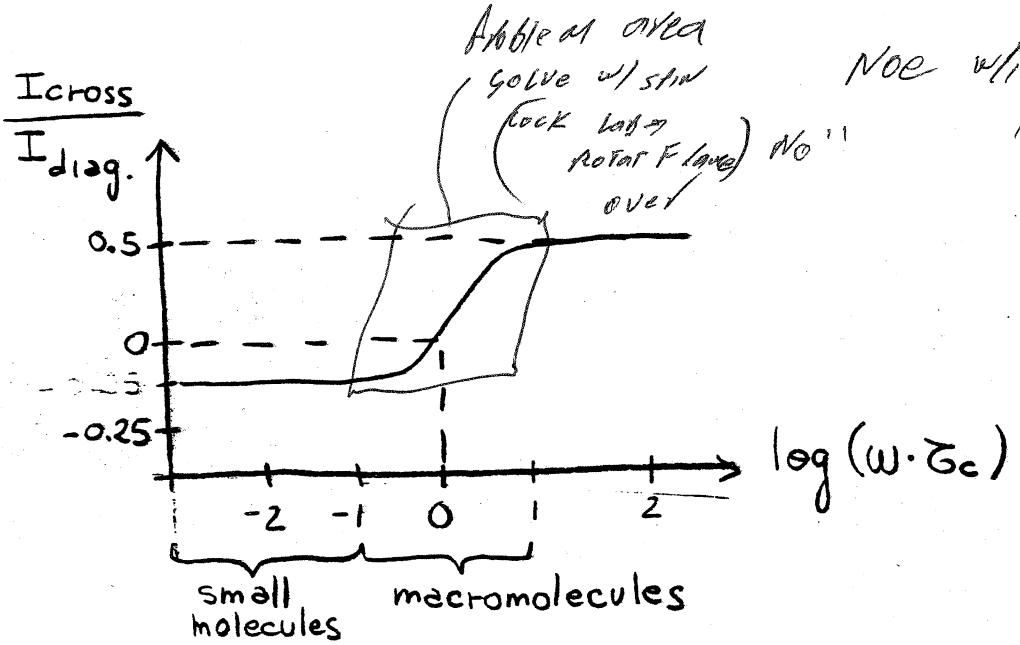
doesn't change rate of long. relax

$I_{1+}S_{2-} \rightarrow I_{1-}S_{2+}$ This does change sign

* move π pulse randomly w/ count (btw π/2's) & cancel J-peaks)

then the modulation of these peaks becomes $\cos\left[(\omega_I - \omega_S)(\tau_m - 2\Delta)\right]$ a random number whose average is zero.

ii) Another problem: the cross peak changes sign at a certain correlation time

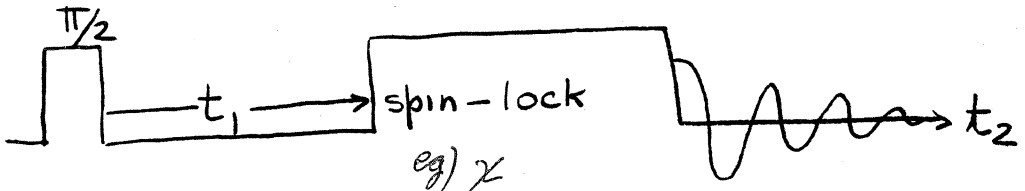


The NOESY experiment is therefore of limited usefulness for macromolecules, where cross peaks may be very small or undetectable.

From a 5 Å resol w/ one NOE peak

* But superb determine w/ 1000's of NOE peaks
∴ limit & limit structure to within 1 Å resol.

A solution to this problem is to carry the NOESY experiment into the rotating-frame, relying on $T_{1\rho}$ cross-relaxation instead of on T_1 effects. The resulting **R**otating-frame nuclear **O**verhauser **E**ffect **S**pectroscop**Y** (**ROESY**) pulse sequence:



eg) χ

1:) Loose γB_1 1/10 inhomog ; ie very long Fast decay

also 1×10^{-9} inhomog

It can be shown that cross-peaks in ROESY are **always** negative with respect to the diagonal. This makes ROESY the preferred technique for medium-sized molecules: like noesy but in rotating frame

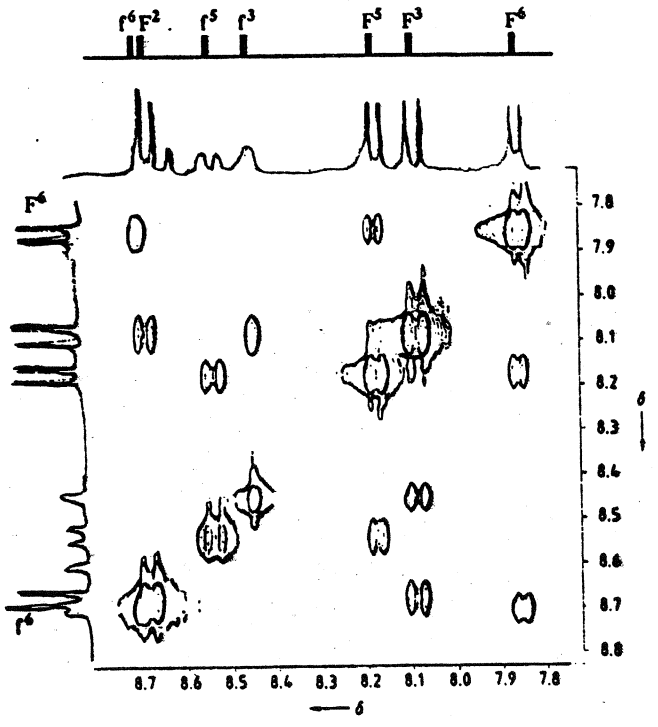
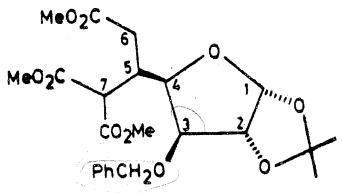


Fig. 40. Section from a 300-MHz ROESY spectrum of *cyclo*(-D-Pro¹-Phe²-Phe³-Pro⁴-Phe⁵-Phe⁶-) in [D_6]DMSO, 320 K, β pulse angle 24°, mixing time 200 ms. The molecule is present in two conformations. The exchange between them is evident from the positive cross signals (black). NOE effects between NH signals give rise to negative (red) cross signals (F^2-F^3 and F^5-F^6). Phenylalanine = F, f (the major isomer is labeled with capital letters).

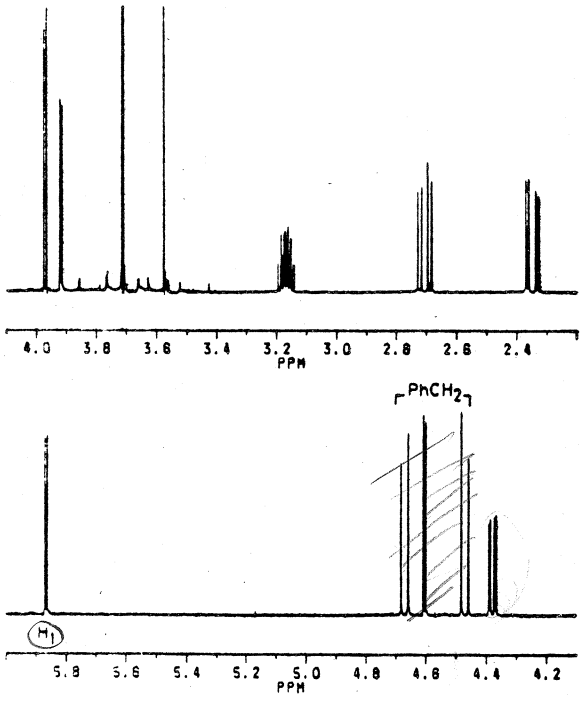
VI.17 PROBLEMS

1) **H,H-COSY:** Analyze the complete time evolution of the density matrix terms arising from spin 1 (i.e., starting from $\rho_0' = I_{z_1}$) in a $(\pi/2)_y$-t_1-$(\pi/2)_y$-t_2 COSY experiment, for a pair of weakly-coupled spins. Classify the resulting operators into populations, zero-quantum, single-quantum (in- and anti-phase) and double-quantum coherences. Calculate the final transfer function arising from these terms. Using symmetry arguments calculate the transfer functions that will arise in the same experiment from spin 2. Indicate which terms will originate peaks in the actual NMR experiment; schematize the total 2D COSY NMR spectrum and the line shape of the peaks in it.

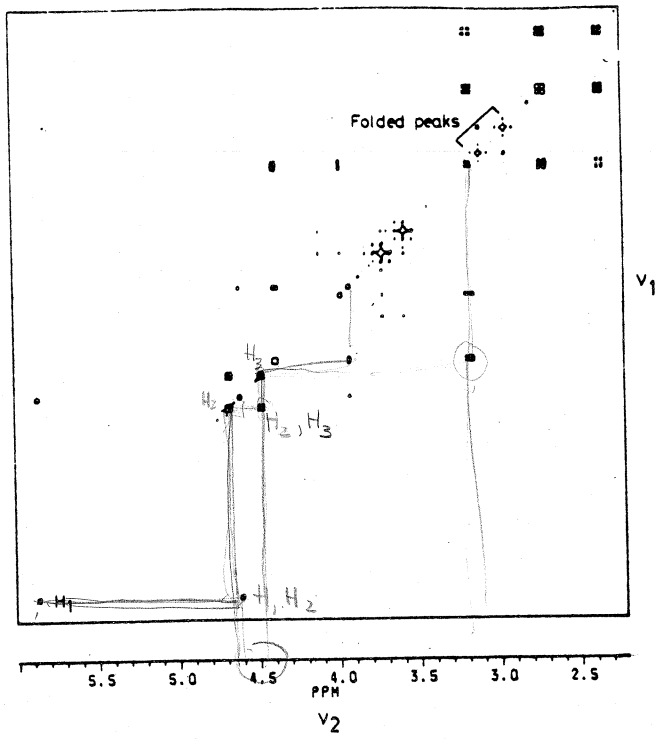
2) For the following molecule:



assign the origin of the different peaks in the 1D NMR spectrum



using the connectivities established by the COSY spectrum:



Justify.

3) i) Given a pair of spins I-S, expand the following spherical operators in terms of the corresponding cartesian operators

$I_+ =$

$I_+ S_0 =$

$I_+ S_- =$

$I_+ S_+ =$

ii) Calculate the evolution of the operators in i) under the effects of a chemical shift Hamiltonian $\mathcal{H}_{CS} = -\omega_S S_z - \omega_I I_z$

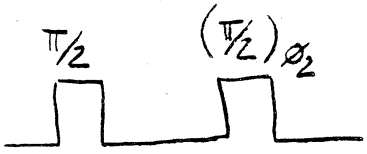
iii) Calculate the evolution of the operators in i) under the effects of a J-coupling Hamiltonian $\mathcal{H}_J = J I_z S_z$

iv) Calculate the evolution of the operators in i) after applying a $\pi/2$ rf pulse along the x-axis. ON I & S? or Just I?

Express all the results in terms of spherical operators.

4) Calculate the effects that a ϕ-rotation around the z-axis ($R_z(\phi) = e^{-i F_z \phi}$) has on all the 16 elements of the spherical operator basis set for a pair of spins I-S.

5) Given 3 coupled spins and a two $(\pi/2)$-pulses sequence



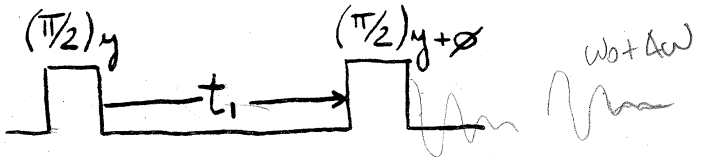
Describe the phase cyclings of ϕ_2, and the treatment of the signal required for selecting the following coherences at the end of the sequence:

$p_{final} = -2, 0, 2$
$p_{final} = -3, -1, 1, 3$

6) Given a state evolving as an I_+S_z coherence, find the transfer functions to all the new coherences that are created by a $(\pi/2)_x$ pulse.

7) Demonstrate that given a multispin coherence, π-pulses are the only type of rf irradiation that do not create new coherences (they just invert the sign of the existing ones).

8) **Coherence pathways in 2D NMR:** Calculate how the data sampled by the two ADC's of an NMR spectrometer in the following experiment:

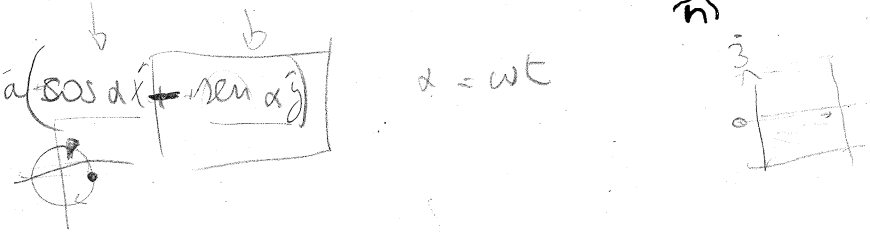


have to be rearranged in order to get a 2D COSY NMR spectrum with quadrature detection along ν_1.

i) For the case $\phi = 0, 2\pi/3, 4\pi/3$

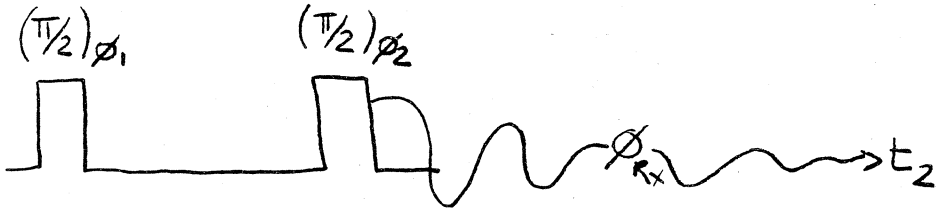
ii) For the case $\phi = 0, \pi/2, \pi, 3\pi/2$

iii) How should the data from experiment i) have to be rearranged in order to collect the echo coherence trasfer pathway?



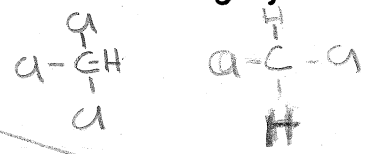
9) given two sets of time-domain 2D COSY data, one in which the echo and the other in which the antiecho pathways were acquired, calculate the data processing necessary for retrieving purely-dispersive NMR line shapes.

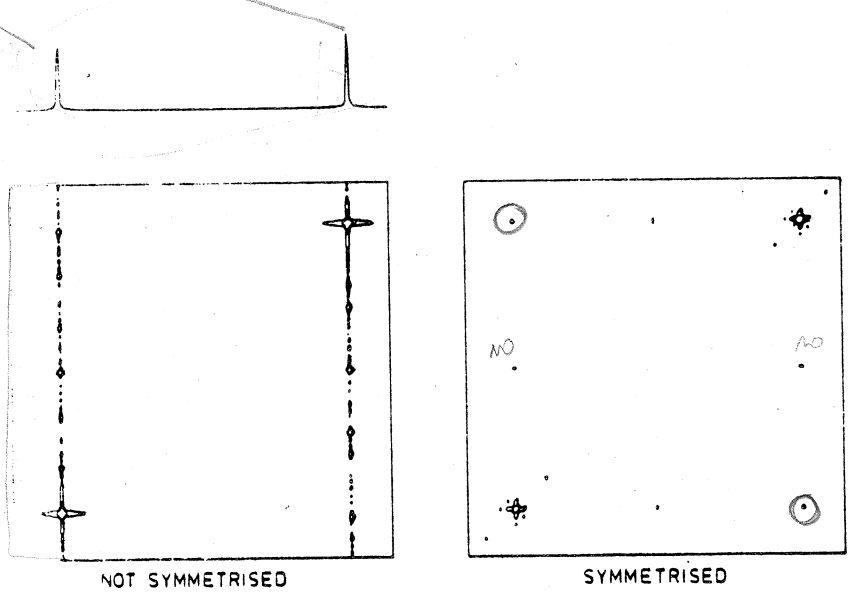
10) **Phase-cycling in 2D NMR:** Calculate the phases ϕ_1, ϕ_2, and ϕ_{Rx}



required for acquiring a phase-sensitive 2D H,H-COSY data set with quadrature detection along ν_1. Specify the coherence transfer pathway collected in each experiment.

11) Mark the cross peaks in the following symmetrized 2D COSY NMR spectrum of a $CHCl_3$, CH_2Cl_2 mixture.

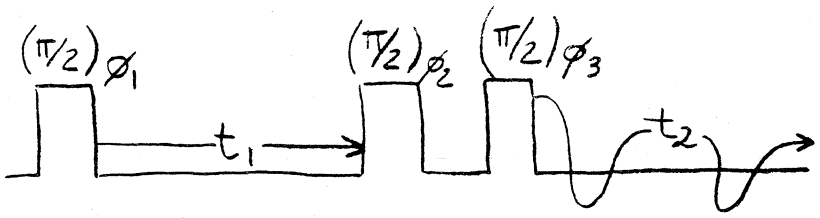




NOTE:

- In COSY experiments cross-peaks are due to J-couplings
- EQUIVALENT NUCLEI CANNOT BE J-COUPLED

12) **DQF COSY:** For the following NMR sequence:



i) Write the transfer function that characterizes the line shapes of diagonal- and cross-peaks arising from a pair of coupled spins (assume for the sake of simplicity $\phi_3 = x$; ϕ_1 and ϕ_2 chose so as to cycle out everything except

$$\ell_{mixing} = \frac{I_{1+}I_{2+} - I_{1-}I_{2-}}{2}$$

ii) Calculate the ϕ_{Rx} involved in the first 16 experiment of the phase cycle, where $\phi_1 = 0$; $\phi_2, \phi_3 = 0, 1, 2, 3$.

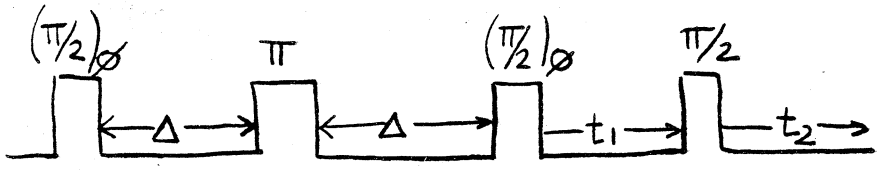
iii) Given 3 coupled spin-1/2 I_1, I_2, I_3; J_{12}, J_{13}, J_{23} non-zero; calculate the transfer functions originating the cross-peak at (ν_2,ν_3) in a DQF COSY experiment. Schematize the phases of the multiplet observed in the spectrum.

13) i) Demonstrate that phase increments in the last rf pulse of a TQF-COSY have to be 0, π/3, 2π/3, π, 4π/3, 5π/3.

ii) Calculate the coherence transfer functions of diagonal- and cross-peaks in TQF-COSY.

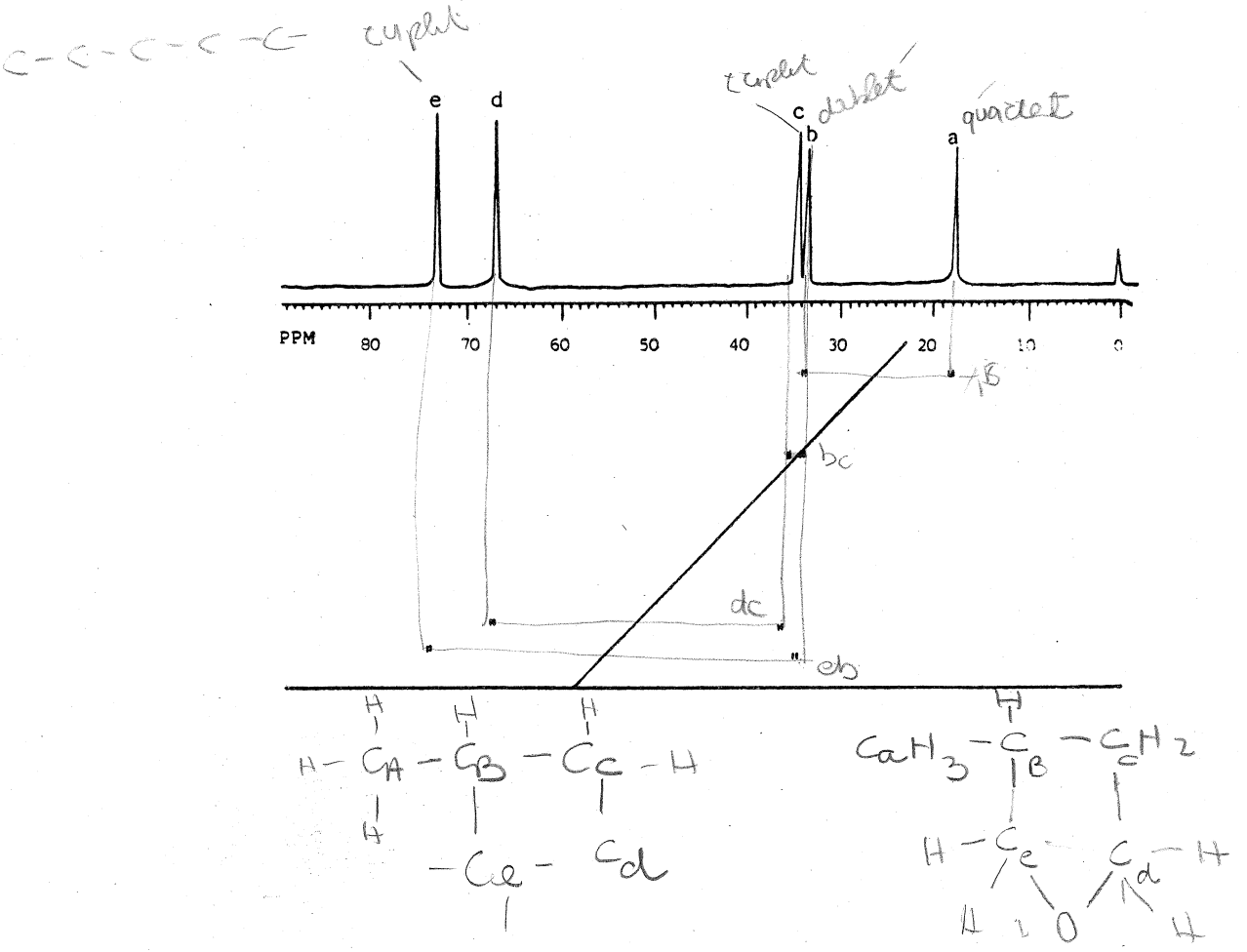
14) Calculate the efficiency of the coherence transfer process among **indirectly** coupled spins in a relayed COSY experiment, as a function of the mixing time Δ.

15) **The INADEQUATE experiment:** Given the basic INADEQUATE pulse sequence

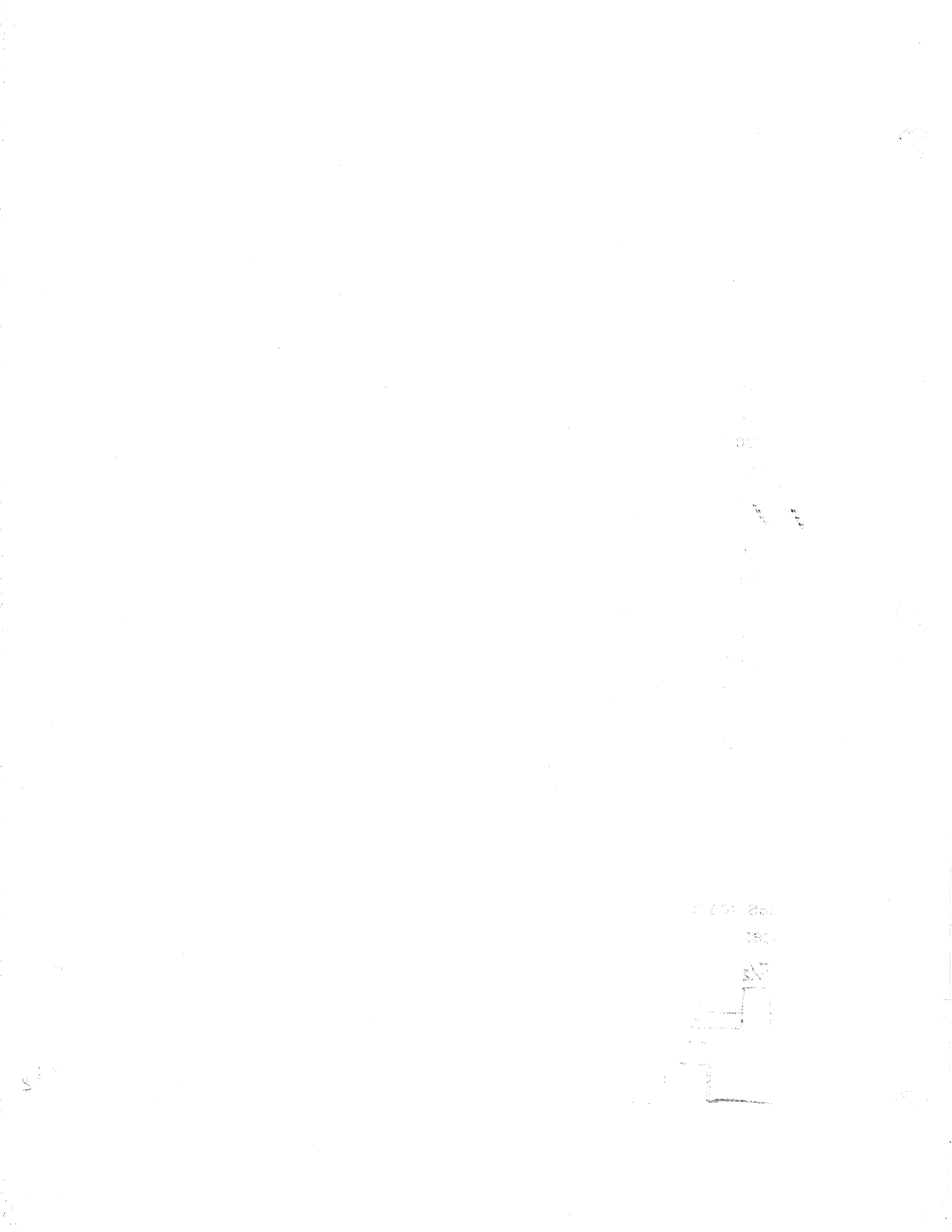


i) Calculate the receiver phase required for observing the evolution of double-quantum coherences during t_1.
ii) Calculate the optimum duration of Δ for a given J.
iii) Calculate how many peaks will appear if this experiment is carried out on a 3 spin homonuclear system I_1-I_2-I_3 in which $J_{12} = J_{23} = J$, $J_{13} = 0$. Specify the resulting line shapes.
iv) Schematize the spectrum arising from a 4 carbon linear chain.

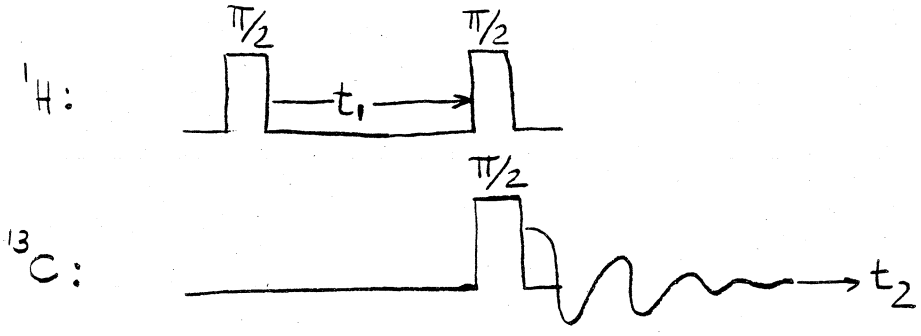
16) A compound with molecular formula $C_5H_{10}O$ affords the following 1D and 2D INADEQUATE ^{13}C NMR spectra:



Upon recording a ^1H-coupled ^{13}C NMR spectrum, peak a appears as a quartet, peak b as a doublet and peaks c-e as triplets. Deduce the structure of the compound.



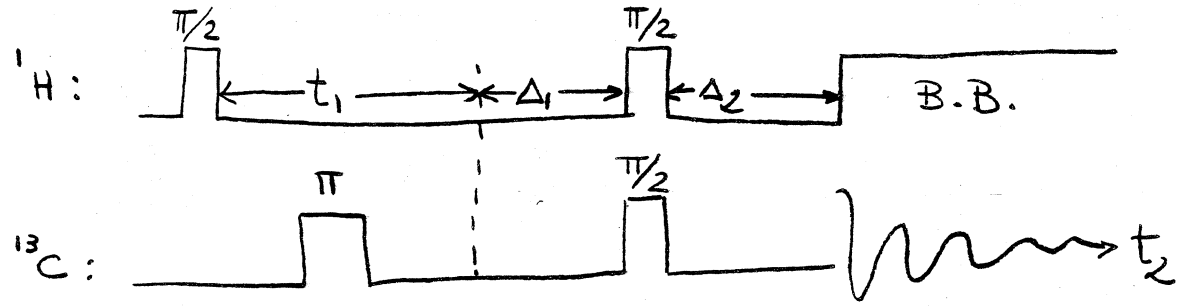
17) **Heteronuclear COSY:** Given the basic H,C-COSY pulse sequence



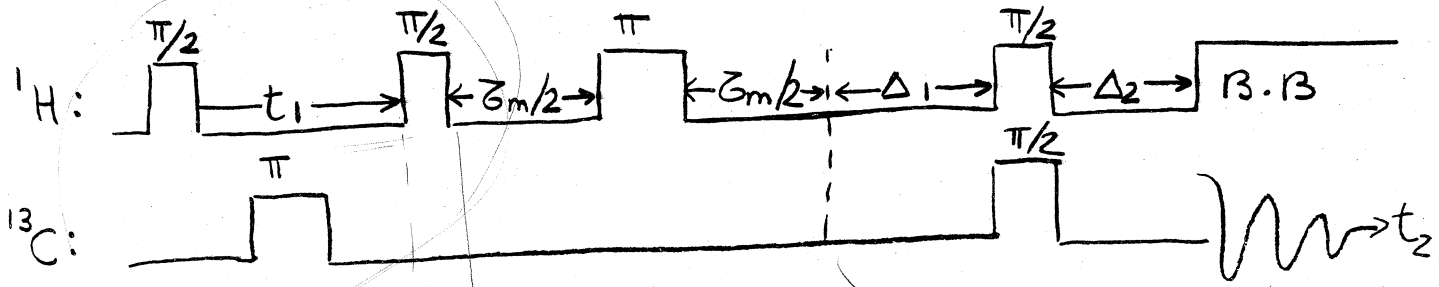
i) Evaluate phases of the rf pulses and of the receiver required to obtain a 2D NMR spectrum with t_1-quadrature detection and with absorptive line shapes.
ii) Describe the 2D NMR spectral line shapes afforded by such an experimet.

18) Given an isolated ^{13}C-^1H pair of spins with chemical shifts ω_C,ω_S and coupling J, calculate the transfer function originating the cross-peak among these nuclei in the 2D-INEPT COSY and in the 2D-DEPT COSY.

19) **Heteronuclear relayed COSY:** Whereas the normal INEPT-derived H,X-COSY experiment



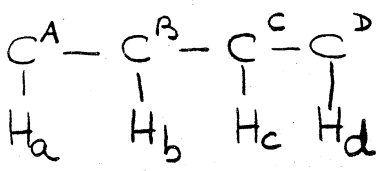
correlates resonance between directly-coupled spins, the H-relayed H,X-COSY sequence:

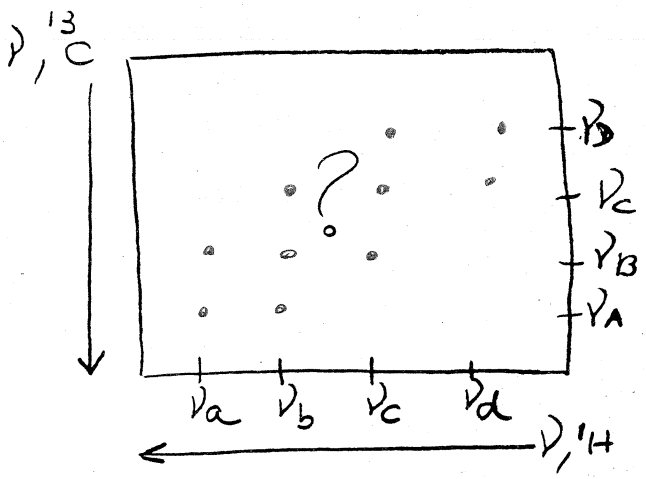


gives cross-peaks among a ^{13}C and a **system** of coupled protons.

i) Explain how the modified pulse sequence works.

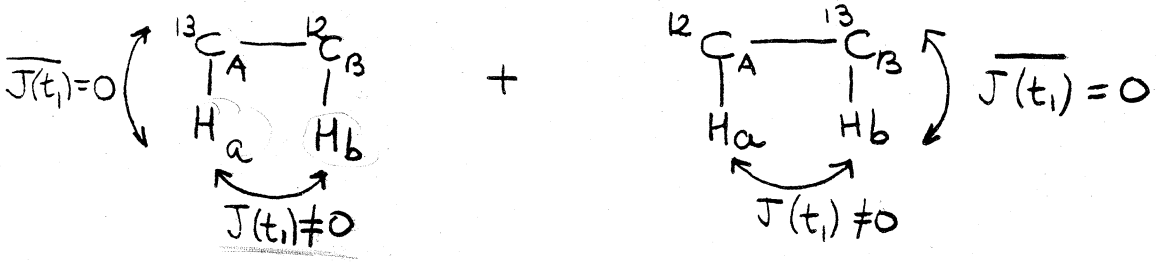
ii) Compare the spectra that would arise from the normal and from the relayed experiment for the system



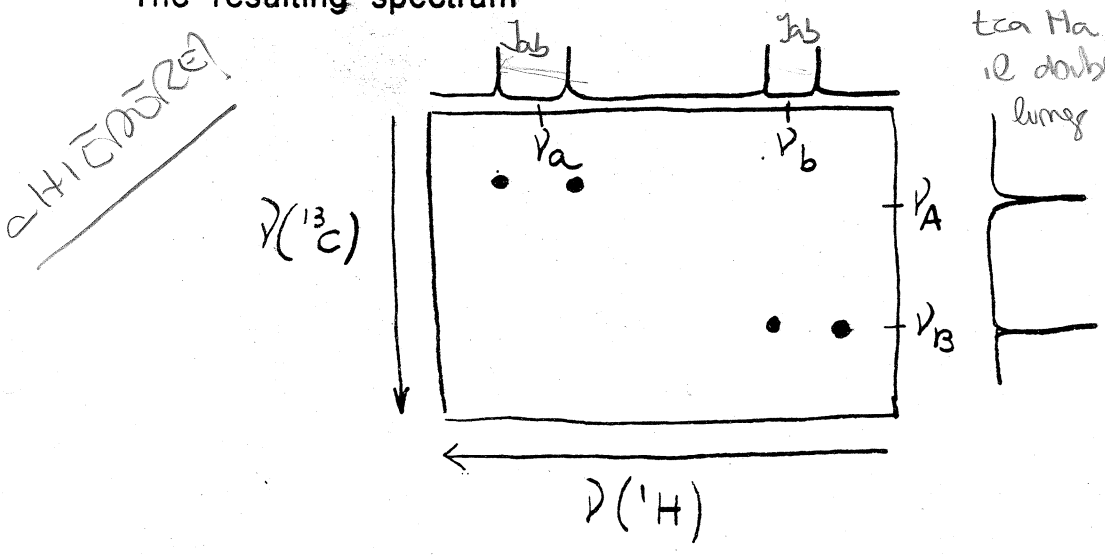


20) **Homonuclear ^1H decoupling in H,X-COSY:** Although the $(\pi)_{^{13}C}$ pulse in the center of the t_1 evolution achieves heteronuclear decoupling along the ^1H dimension of the H,X-COSY, couplings between the ^1H coupled to the ^{13}C and its neighboring protons still show in the spectrum.

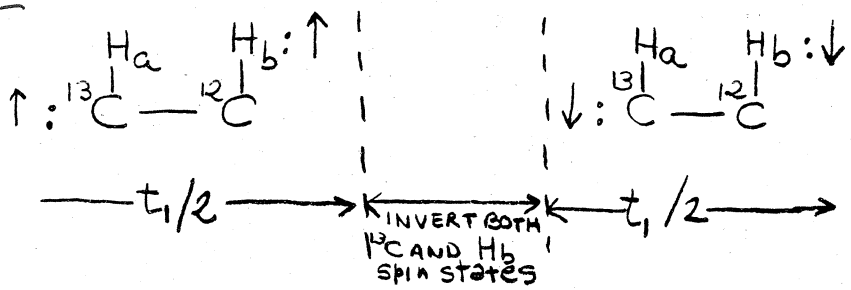
The system under observation



The resulting spectrum

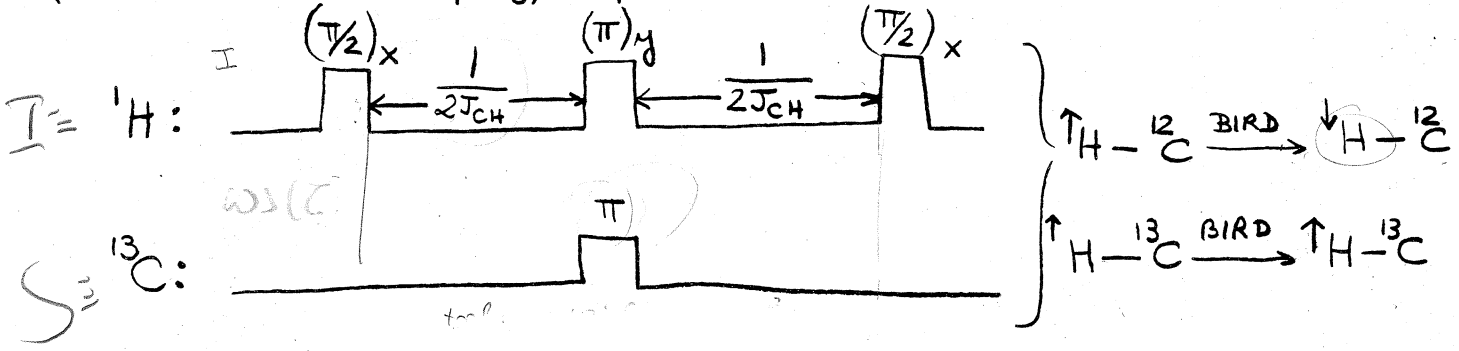


It is possible to achieve homonuclear decoupling during t_1 by inverting the spin state of all the protons not bonded to a ^{13}C at the middle of the evolution period ($t_1/2$):



Evolution of H_a spins is exclusively due to its chemical shift

A ^1H π-pulse selective to the carbon spin can be implemented using the **BIRD** (**BI**linear **R**otation **D**ecoupling) sequence



i) Explain how the BIRD sequence works.
ii) Show how BIRD can be included in the standard INEPT-type H,X-COSY sequence to yield the ν_1-decoupled experiment.

21) i) Calculate the transfer function originating H-X cross peaks in the basic HMQC pulse sequence used for inverse spectroscopy.
ii) How would you modify this pulse sequence to include in it a BIRD-type selection of the protons bonded to active X spins?

22) Calculate
i) the complete phase-cycling of the rf pulses and receiver
and
ii) describe in detail the data processing
involved in the acquisition of purely-absorptive 2D exchange NMR spectra with quadrature detection in uncoupled spin systems (Ernst. Ch. 9).

23) In the COCONOESY (COmbined COsy and NOESY) experiment two sets of 1D data are acquired *per scan*; processing of these data sets gives a 2D COSY and a 2D NOESY spectrum. How would you implement this experiment using the standard 3-pulse 2D exchange sequence? What would be the limitations of such an experiment?

24) Explain the origin of the diagonal peaks and the dependence of the cross peaks intensity with mixing time in the following ^{119}Sn NOESY NMR spectra of a 1:1 $SnCl_4$:$SnBr_4$ solution.

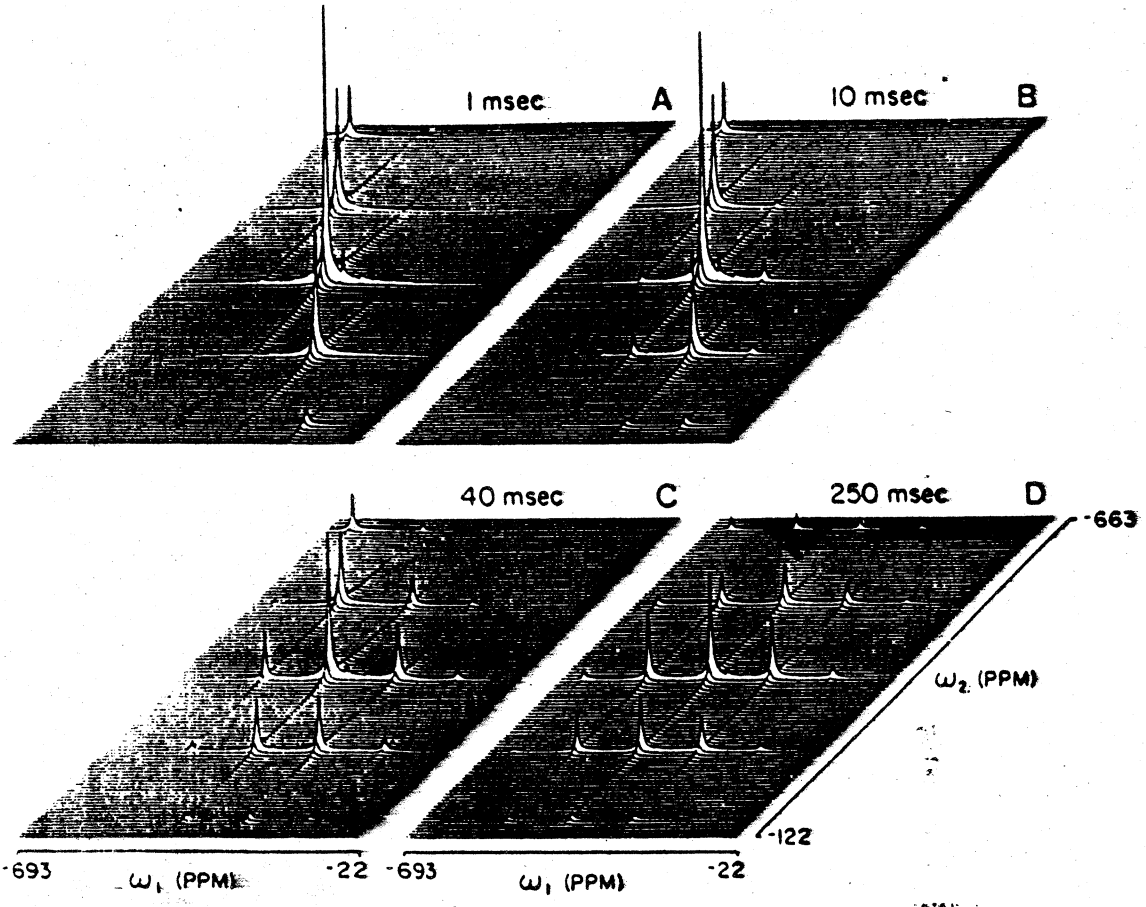
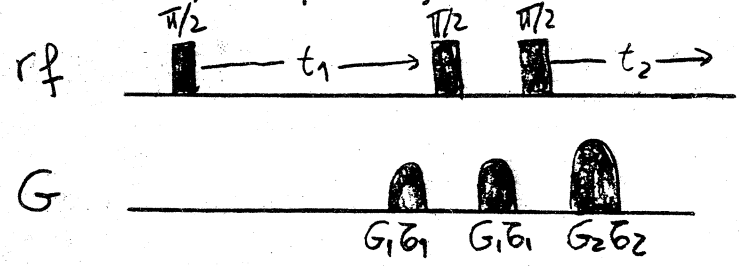


FIG. 1. Tin-119 2D absolute-value-mode NMR spectra of a 1:1 *M* mixture of $SnCl_4$ and $SnBr_4$ at 340 K and 186.4 MHz, as a function of the mix time, τ_m.

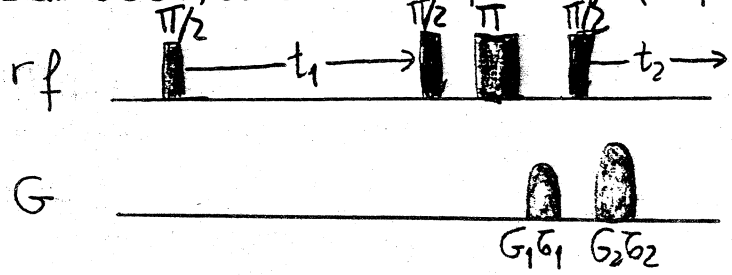
(or intensities)

25) Explain how the following gradient-enhanced sequences achieve the desired coherence transfer selection, specifying the lengths that the gradient pulses should have and the coherence transfer diagrams

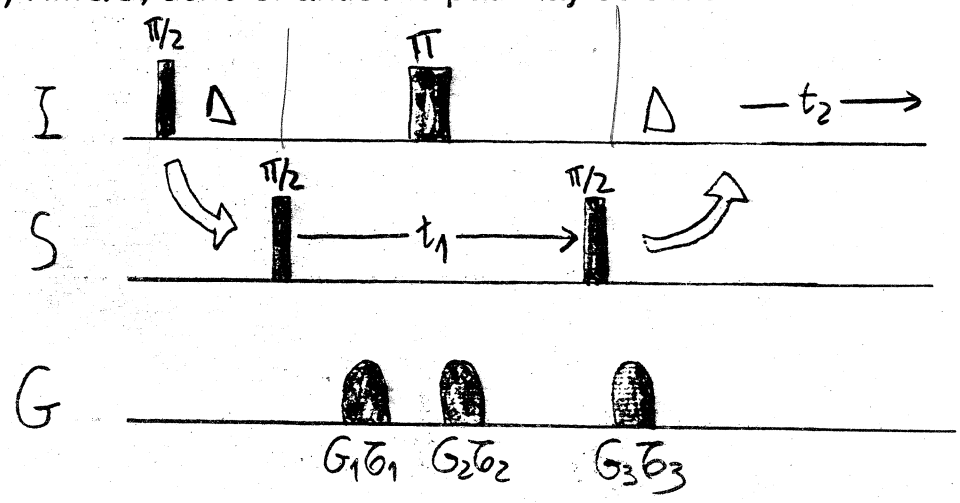
i) DQF-COSY; echo pathway selection



ii) DQF-COSY; echo+antiecho pathways (amplitude modulation)



iii) HMQC; echo *or* antiecho pathway selection



$I_z \rightarrow I_x \rightarrow 2I_yS_z \xrightarrow{1/2} 2I_yS_y \longrightarrow -2I_yS_z$

CS

^{13}C