---------- **Chapter 13: Homonuclear AX Experiments (387-432) ------- 45 p ---------- ###**

There are four major sections in this chapter. each illustrates a different "experiment".
A. COSY
B. INADEQUATE
C. INEPT
D. BICELLES

A. COSY

13.1 COSY (387-399) This is a very complex section, I have lots of notes therefore.

I have now also a completely separate document COSY.doc where I do the theory in a systematic manner. I recommend reading those notes and not bothering with these original notes here. See also metareview.

13.1.1 The Assignment Problem. Suppose you a sample with two different AX systems and each one makes 4 peaks for a total of 8 peaks. How would you interpret the spectrum shown? There are three possibilities, you have no way to know which is right! This is "the assignment problem".

13.1.2 COSY pulse sequence (COSY = correlation spectroscopy). The solution to the above problem is to get a 2D Fourier spectrum and then look at the peaks as on page 389. Notice how these 2D's are all different, so you can solve your assignment problem if you do COSY. Invented by Jean Jeener in 1971, so not late-breaking news.

The method is to do two different experiments and combine the results using the States method which we read about in an earlier chapter. The two pulse sequences are shown page 390 top.

13.1.3 Theory of COSY. I agree that the $(\pi/2)_x$ pulse will flip I_z down to $-I_y$ because we have done that lots of times before. But suddenly Levitt is doing weird things. First, the TE matrix ρ really has a unity element in it and a factor 1/4 as on page 366. But here he is omitting the unity and this factor of 4 without saying he is doing that, though this gets mentioned much later.

The next strangeness is his claiming he can write these I_y terms in the bizarre way shown page bottom p 390. This is totally non-obvious and author is totally mum. He knows something that he failed to teach us along the way, so now I have to wander off and do it myself.

Question: Where does the bottom line on page 390 come from?

Here is a reasonable starting point: $-I_{1y} - I_{2y} = -(1/2i)[I_1^+ - I_1^-] - (1/2i)[I_2^+ - I_2^-]$

As usual, these are meant as operators in the direct product space. One can always say $1_1 = I_1^\alpha + I_1^\beta$ which just says the sum of all the projection operators is unity (in that space). See also page 165 showing this is true. So we can then rewrite the above as:

$$= -(1/2i)\, I_1^+ I_2^\alpha - (1/2i)\, I_1^+ I_2^\beta - (1/2i)\, I_2^+ I_1^\alpha - (1/2i)\, I_2^+ I_1^\beta$$

$$+ (1/2i)\, I_{1}^{-}\, I_{2}^{\alpha} + (1/2i)\, I_{1}^{-}\, I_{2}^{\beta} + (1/2i)\, I_{2}^{-}\, I_{1}^{\alpha} + (1/2i)\, I_{2}^{-}\, I_{1}^{\beta}$$

So yes, we can write our $-I_{1y} - I_{2y}$ as the sum of these 8 terms. You can now see that each term IS one of the 8 single-quantum coherences in the ρ matrix on page 344. Each of the above term has only a single non-zero matrix element in the direct product space! For example [α is the upper state, β the lower]

$$\langle \beta\, \alpha \,|\, (1/2i)\, I_{1}^{-}\, I_{2}^{\alpha} \,|\, \alpha\, \alpha \rangle = (1/2i) \langle \beta | I_{1}^{-} | \alpha \rangle_{1} \langle \alpha | I_{2}^{\alpha} | \alpha \rangle_{2} = (1/2i) * 1 * 1 = (1/2i)$$

The left state determines the matrix row, the right state the column*, so this is row 3, col 1, which is the -α element of ρ. [Note that our I_y stuff contains ONLY single-quantum coherences]

* Why is this true? This is how the elements are defined! For example, if you put column vector (1000) on the *right* of the ρ matrix, which is state αα, you pick off the first *column* of the matrix. That is why we associate the right state with the column. And close with row vector (0100) and you pick off the second row, so the left state (the "bra") is the row. I cannot help feeling Malcolm could not have done a cleaner job explaining his box notation.

So, what we learn is this: the initial $(\pi/2)_x$ pulse takes your TE state which had no coherences, and it creates a ρ matrix which has 8 single-quantum coherences that are all the same size, but of course half of them are the CC of the other half, as our results above show. This is what the drawing on page 391 middle means. We have "activated" all 8 SQ coherences by the same amount!

After this, we just let things propagate as shown page 391 for time t_1. We derived these facts back on page 352, so each coherence gets an appropriate energy value. Then I agree with the bottom equation, where I now see that the pair of operators just defines a "1" somewhere in the 4x4 ρ matrix. And the labels on them are the same as the labels on the ρ element -- THAT is the box notation! So I am now very happy with page 391.

He shows free propagation as horizontal lines, top page 392. We than slap ρ with our second pulse. Malcolm now does a brute force calculation to see what this does to each of our 8 terms. He computes it just for our one term, the -α term. The second pulse blows EACH of our 8 coherence terms into equal amounts of all 16 ρ elements! So if we only care about the single-quantum guys, we get the secondary branching shown top of page 393. Fine. This is all an example of a pulse doing "coherence transfer".

We are now going to let things propagate for time t_2 but he does not bother to show that top page 393.

What is the size of ρ matrix element "α - " at time $t_2 = 0$? I think you have to add up all the paths, and on page 393 he shows only one of the paths ending in "α - " ρ element. We have the first two factors coming from the first pulse and then quiet propagation, then we get the (-1/4) from the second pulse, then again we have quiet propagation but this time at the "- α" frequency. The final 2i comes from page 587 which I agree with.

Comment: Now look at what has happened. The first propagation was phasing at the first Ω for time t_1 and the second propagation was phasing at the second Ω for time t_2. Recall from page 355 what the various spectral lines look like and why there are 4 and not 8. The Ω values are shown on page 352. So the term shown is going to show up in the double Fourier spectrum at $\Omega_1 = -\alpha$, and $\Omega_2 = \alpha-$, and this spectral peak is then shown page 394.

Now, to get the total value of the final α-, I think you have to add up all the possible paths including the + and - intermediate guys, probably they just double the results. If we just ignore the + stuff, we would conclude that there are four intermediate - paths to final ρ element "α -". Each has a frequency on its first leg and the same for the second leg, so these would be the four peaks in the Ω_2 row. So fine, you end up with 16 peaks! For each AX system, you will get 16 different peaks. This is shown on page 395 top where we have two such systems.

The 16 peaks are divided into four sets. Two of them are cross peaks, two sets are diagonals that are of less interest we shall see.

So, we finally see why you might possibly want to use a 2D Fourier spectrum in NMR!

Top page 395 does bring up a question: why don't the two AX systems interact with each other? Presumably we have two pairs of coupled spins so a-x and A-X, and a is not coupled to X, for example. In this case, the Hamiltonian is additive and everything follows through. The two pairs of coupled spins could be in the same or different molecules I think. Certainly if in different, you won't get coupling as was shown back on page 216. If in same, if they are far apart, won't be coupled I would assume.

13.1.4. States Processing (395). So far we got to the bottom equation on page 393. We know that the 2D Fourier of this looks like the right side of the top figure on page 112. We know that each peak has a real part like that shown page 113, which is not all that clean and has long tails. The imaginary part is the horrible phase twist peak shown on page 114 top. All this comes simply from multiplying two complex Lorentzians as done on page 113. The complaint is that each peak in your 2D spectrum has this messy stuff and the long "tails" or "folds" of the peaks tend to overlap and wash out the picture.

This is where States comes in, and here we go through the details. We now want to go back to the simpler formalism we used in chapter 12.

time 1 = TE $\rho = (1/4)\,1 + (1/4)B\,(\,I_{1z} + I_{2z}\,)$

time 2: $\rho = (1/4)\,1 + (1/4)B\,(\,-I_{1y} - I_{2y}\,)$ // after the first π/2 pulse

Now we need to propagate this thing for time t1 including the J coupling. We have to sandwich three groups of rotation operators in the order of our choice, as shown page 374. Let's try this imitating page 380.

Work first on I_{1y}. The Ω_2 rotation does nothing, so we start the same way. But now we do the Ω_1 rotation about z. Our rule for this comes from page 375 and we get,

$$I_{1y} \rightarrow I_{1y}\cos(\Omega_1 t_1) - I_{1x}\sin(\Omega_1 t_1)$$

Now we finish off doing the "J rotation" which is that shown in 12.23 page 374. We have to rotate using the operator $2I_{1z}I_{2z}$ and this is *not* the product of two z-rotations (that would be if you had the sum). This is where we get involved with the tricky diagrams on page 377. They show what such rotations do to various things, and I will now install those things:

$$\rightarrow [\, I_{1y}\cos(\pi J t_1) - 2I_{1x}I_{2z}\sin(\pi J t_1)\,]\cos(\Omega_1 t_1) - [\, I_{1x}\cos(\pi J t_1) + 2I_{1y}I_{2z}\sin(\pi J t_1)\,]\sin(\Omega_1 t_1)$$

Now how can we quickly get the I_{2y} result ? I think just do $\Omega_1 \rightarrow \Omega_2$ and $I_{1y,x} \rightarrow I_{2y,x}$ in the above! But then looking at page 377, we also have to switch x ↔ z on the double operator pairs. So:

$$+ [I_{2y} \cos(\pi J t_1) - 2I_{1z}I_{2x} \sin(\pi J t_1)] \cos(\Omega_2 t_1) - [I_{2x} \cos(\pi J t_1) + 2I_{1z}I_{2y} \sin(\pi J t_1)] \sin(\Omega_2 t_1)$$

Now let's start with $- I_{1y} - I_{2y}$ and add an overall minus sign so we have:

$$- I_{1y} - I_{2y} \rightarrow$$

$$- [I_{1y} \cos(\pi J t_1) - 2I_{1x}I_{2z} \sin(\pi J t_1)] \cos(\Omega_1 t_1) + [I_{1x} \cos(\pi J t_1) + 2I_{1y}I_{2z} \sin(\pi J t_1)] \sin(\Omega_1 t_1)$$

$$- [I_{2y} \cos(\pi J t_1) - 2I_{1z}I_{2x} \sin(\pi J t_1)] \cos(\Omega_2 t_1) + [I_{2x} \cos(\pi J t_1) + 2I_{1z}I_{2y} \sin(\pi J t_1)] \sin(\Omega_2 t_1)$$

Now I will move the right side trig functions to right after their operators:

$$- [I_{1y} \cos(\Omega_1 t_1)\cos(\pi J t_1) - 2I_{1x}I_{2z} \cos(\Omega_1 t_1)\sin(\pi J t_1)]$$
$$+ [I_{1x} \sin(\Omega_1 t_1)\cos(\pi J t_1) + 2I_{1y}I_{2z} \sin(\Omega_1 t_1)\sin(\pi J t_1)]$$

$$- [I_{2y} \cos(\Omega_2 t_1)\cos(\pi J t_1) - 2I_{1z}I_{2x} \cos(\Omega_2 t_1)\sin(\pi J t_1)]$$
$$+ [I_{2x}\sin(\Omega_2 t_1) \cos(\pi J t_1) + 2I_{1z}I_{2y} \sin(\Omega_2 t_1)\sin(\pi J t_1)]$$

and finally do out the signs and we obtain ρ at time 3:

$$- I_{1y} \cos(\Omega_1 t_1)\cos(\pi J t_1) + 2I_{1x}I_{2z} \cos(\Omega_1 t_1)\sin(\pi J t_1)$$
$$+ I_{1x} \sin(\Omega_1 t_1)\cos(\pi J t_1) + 2I_{1y}I_{2z} \sin(\Omega_1 t_1)\sin(\pi J t_1)$$

$$- I_{2y} \cos(\Omega_2 t_1)\cos(\pi J t_1) + 2I_{1z}I_{2x} \cos(\Omega_2 t_1)\sin(\pi J t_1)$$
$$+ I_{2x}\sin(\Omega_2 t_1) \cos(\pi J t_1) + 2I_{1z}I_{2y} \sin(\Omega_2 t_1)\sin(\pi J t_1)$$

and so we have now verified (13.1) at the bottom of page 395.

Now we need to apply a $(\pi/2)_x$ pulse to this mess. This is just going to permute the operators according to our pictures. Here is a list of what will happen:

First, in either subspace:

$$I_x \rightarrow I_x \qquad I_y \rightarrow I_z \qquad I_z \rightarrow - I_y$$

Let's just edit in these changes in each subspace, because this is not a product operator like we had in the J case, we just have a product of rotation operators in the two subspaces:

$- I_{1z} \cos(\Omega_1 t_1)\cos(\pi J t_1) - 2I_{1x}I_{2y} \cos(\Omega_1 t_1)\sin(\pi J t_1)$	0	2,0
$+ I_{1x} \sin(\Omega_1 t_1)\cos(\pi J t_1) - 2I_{1z}I_{2y} \sin(\Omega_1 t_1)\sin(\pi J t_1)$	1	1
$- I_{2z} \cos(\Omega_2 t_1)\cos(\pi J t_1) - 2I_{1y}I_{2x} \cos(\Omega_2 t_1)\sin(\pi J t_1)$	0	2,0

$+ I_{2x}\sin(\Omega_2 t_1)\cos(\pi J t_1) - 2I_{1y}I_{2z}\sin(\Omega_2 t_1)\sin(\pi J t_1)$ 1 1

and this agrees with the top equation on page 396.

Now let's classify the above 8 terms in terms of quanta-change (marked on right). We know, for example, that I_{1z} has diagonal matrix elements, so 0 quanta change. And $I_{1x}I_{2y}$ when you put both in raise and lower sum requires two quanta change (or zero). And so on. So we keep only the 1 quanta stuff since only that stuff affects NMR signal.

$+ I_{1x}\sin(\Omega_1 t_1)\cos(\pi J t_1) - 2I_{1z}I_{2y}\sin(\Omega_1 t_1)\sin(\pi J t_1)$ 1 1

$+ I_{2x}\sin(\Omega_2 t_1)\cos(\pi J t_1) - 2I_{1y}I_{2z}\sin(\Omega_2 t_1)\sin(\pi J t_1)$ 1 1

[Digression: But this logic is wrong! You have to propagate through time t_2 to see what really happens to terms. Let's make a little list:

I_{1z} Ω_1 does nothing, Ω_2 does nothing , J does nothing, stays as I_{1z} so has no single-quantum, so throw this term out (it is a zero-quantum term in the end).

I_{1x} Ω_1 keeps in class, Ω_2 does nothing, J creates mix of same and things like $I_{1y}I_{2z}$. All the resulting terms are single-quantum, so keep.

$2I_{1x}I_{2y}$ Ω_1 keeps in class, Ω_2 keeps in class, J does nothing according (12.26) last, so this operator "stays as is" which means it stays as a double-quantum, so we throw it out.

Similar arguments for the other right-side terms in 396, they all go out.]

Now use trig identities on the trig products above, to wit,

$\sin(\Omega_1 t_1)\cos(\pi J t_1) = (1/2)\ \{\sin((\Omega_1 - \pi J)t_1) + \sin((\Omega_1 + \pi J)t_1)$

$\sin(\Omega_1 t_1)\sin(\pi J t_1) = (1/2)\ \{\cos((\Omega_1 - \pi J)t_1) - \cos((\Omega_1 + \pi J)t_1)$

and similarly for the Ω_2 factors. Following our author, we look first at the last two terms and these give the first line in (13.4) page 396. For the moment, let's accept the ρ^{sin} result. To show this, we have to go back and apply a $(\pi/2)_{y\text{-}bar}$ as our first pulse in place of $(\pi/2)_x$ and that is going to take me a while to do, so again, accept that result for now so we can continue with the States applicability analysis.

So I now assume the second line in (13.4) and have derived the first. Author now makes a series of claims, each of which are not obvious to me:

(1) the terms meet the States requirements. First, notice that the I_{2y} is imaginary, so when you multiply by 2i as on page 587, you get a real a_ℓ , one of the States requirements on page 115. The second requirement is the mirror peak location in the Ω_1 variable, but this is just what the cosine says, and we have two of these cosine terms instead of 1. So I think we meet States requirements.

(2) He says "the term $- 2I_{1z}I_{2y}$ implies an antiphase absorption peak centered at Ω_2" . Well, the fact that I_{2y} is imaginary and we have the receiver's 2i factor means that our signal is real, and this means we have

a normal *absorption* peak. But we have two peaks at $\Omega_1^0 \pm \pi J$, and they have opposite signs, so this is an *anti-phase absorption double peak*. In a quartet, you get this happening in both directions, so you might then call the quartet a "doubly antiphase cross peak". The black and white circles in the page 397 figure are indicating the polarity of the peaks. From his drawing, it is suggested that the black circles are the positive peaks. Read the following for more details on these summary statements.

The minus sign means it points downward instead of up in our little spectra, so that must be the meaning of "*antiphase*". The final propagation period which takes us to time 5 in effect is measured in t_2 with Fourier corresponding variable Ω_2 , so this propagation will have a peak in Ω_2 at the location of whatever coherence we are thinking about.

(3) Now why is this a "cross peak" and not a "diagonal" peak? Notice that (13.2) has four terms, and he is only talking now about the second term which I have surrounded in pencil. This term is I_{2x}-class and has matrix elements of the form $<\alpha\alpha$ ...$\alpha\beta>$ and so is α- type. Within this α- type, we see there will be two distinct peaks at $\Omega_1^0 \pm \pi J$. How do I relate this to the page 397 top figure?? I have a **contradiction** here because along the top of the 2D picture, there is only one peak labeled α-. I think the resolution is this, as described in the next paragraphs: you don't do your 2D FT until you do the t_2 propagation, and that will change the spin operators such that this contradiction will go away. In other words, you have to do the t_2 propagation to find your signal $s(t_1, t_2)$ and only THEN can you do the 2D FT.

Resolving this contradiction. We know that 13.2 is the full ρ single-quantum cos result at time 4 which is after the second pulse and when t_2 propagation begins. What is the effect of t_2 propagation? We saw the effect of t_1 propagation going from (13.1-) to (13.1), *lots of complexity was added*. In the second propagation we expect terms with $\Omega_2^0 \pm \pi J$ peaks in the Ω_2 variable. It is the signal that runs during this last propagation that we are supposed to Fourier transform. The operators are going to get changed from what you see in (13.2). [in general, during the second propagation you could get $\Omega_2^0 \pm \pi J$ peaks *and* $\Omega_1^0 \pm \pi J$ peaks both in the Ω_2 variable conjugate to t_2. This is because during this propagation, you have to do both these single z rotations. But, if we have a term - $2I_{1z}I_{2y}$, we know that the Ω_1^0 z rotation does nothing, so this term will have only $\Omega_2^0 \pm \pi J$ peaks in Ω_2.]

Now consider just the 1 of 4 terms of (13.2) picked out in the upper part of (13.4). This has peaks at $\Omega_1^0 \pm \pi J$ in the Ω_1 variable, no question about that. So when we propagate this term we get peaks $\Omega_1^0 \pm \pi J$ in Ω_1 and peaks at $\Omega_2^0 \pm \pi J$ in Ω_2 [for the reason in square brackets above]. For sure, this represents a *cross peak quartet*! A diagonal quartet would have peaks at $\Omega_2^0 \pm \pi J$ in both Ω_1 and Ω_2, for example. Therefore, equation (13.4) when it is propagated creates a signal whose FT will be the lower-right quartet in the page 397 figure.

So, when Malcolm says "the term - $2I_{1z}I_{2y}$ *implies* an antiphase absorption peak centered at Ω^0_2 in Ω_2" I think he means that, when you propagate this term through t_1, you will pick up $\Omega_2^0 \pm \pi J$ in Ω_2, *and* this operator will transform into an operator that has the right single-quantum matrix elements. Let's think about how the operator - $2I_{1z}I_{2y}$ will propagate. We know that there are 3 rotations to worry about. We know that the single Ω_2 rotation will keep I_{2y} in its "class", just mixing I_{2y} and I_{2x}. The single Ω_1 rotation will do nothing because there is an I_{1z} factor. Now, they key thing is the J rotation which uses operator $2I_{1z}I_{2z}$. From page 377 we know that this will rotate $2I_{1z}I_{2y}$ into some I_{2x} and this will have single-quantum effects. So this is why "the term - $2I_{1z}I_{2y}$ *implies*" something.

There must be a better graphical tool for doing all these propagations and things. Sort of a Feynman diagram set of rules. He has started this a little bit, maybe there will be more.

I see Malcolm's dilemma. To do this right, you really need to propagate out to some time t_2, but then you probably get 64 terms to keep track of.

Now, what about the other three terms in (13.2)? The fourth term has I_{2z} so will have only Ω_1^0 peaks in the Ω_2 variable. But clearly it also has Ω_2^0 peaks in the Ω_1 variable. So (as Malcolm states on page 397), this term gives the other cross peak quartet. Notice it has the same sin*sin form which makes cos - cos.

Now look at the first term in (13.2). It already has Ω_1^0 peaks in Ω_1 . The subsequent Ω_2 sole rotation will do nothing, so it will then have only Ω_1^0 peaks in Ω_2 as well, so this is a diagonal quartet term. As Malcolm says about this situation, "everything is wrong". It is in the cosine States term, but shows sin differences, which we know means trouble. It is imaginary instead of real so has dispersion peaks instead of absorption peaks.

Now, when you do the States procedure on the cross peak quartet signals, you will get the four clean peaks as suggested top of page 397, I completely agree. However, for a diagonal quartet, you expect and in fact get a horrible mess, as shown page 398. As long as this diagonal quartet is "far away" from the cross peak quartet, its long tails won't harm the cross peak data. There is a fancy version of COSY that solves this problem, called double-quantum-filtered COSY, but Levitt is not going to treat this messy thing I am sure. But the regular COSY is now understood, and we have *finally* finished this section!

Question: why does an initial $(\pi/2)_{y\text{-bar}}$ pulse as shown page 390 create our "sine" partner data? Well, that pulse instead of making $-I_{1y} - I_{2y}$ will make $-I_{1x} - I_{2x}$ because we rotate z down around y-bar. This is in some sense the same as just changing to a new coordinate system where $y \rightarrow x$ (unit vector). Such a 90 degree rotation interchanges sines and cosines for single z-rotations, roughly speaking. So that is why in (13.3) we see all single-angle rotation sines and cosines interchanged. There is more to think about, this is not a proof, but if I wanted to take the time to do it all, I could fill out this proof. The interchange is in fact doing cos $\rightarrow$ -sin which is not surprising (part of my "exchange").

13.1.5 Experimental examples (398) . Page 399 shows an example where for an organic molecule with two rings and perhaps ~ 16 H atoms. I wish he said a little more about this picture. Are there some quartets like we have been talking about? Remember that for each J-coupled AX system in here we expect four quartets. Maybe the small squares are really quartets. But the picture is also going to have stuff on the diagonal for non-J coupled chem shifts of H's (I think). I think if you let $J \rightarrow 0$, a cross quartet will vanish due to its anti-phased peaks just canceling each other. So, the existence of the J coupling pulls things off the diagonal axis and lets you see them more clearly!

So I think the point of the page 399 figure is just that doing 2D spectroscopy has certain advantages in general, beyond the specifics of this chapter in its application to the case of one or two AX J-coupled systems. It is like the 2D protein gel Western blot, you can see much better what you have! And you could take this to 3D or 4D in theory, but harder to look at things (but a computer program could look).

The page 400 picture shows COSY for a bio molecule (this one is double filtered). As expected, since there are lots of H atoms in a 120 AA protein, you get lots of peaks in the picture, so in this case the 3D and 4D must be done. You want to pull the dots apart, but not clear to me how this would work. How

would we extend the presentation of this chapter on a single AX J-coupled system to 3D? We would do another pulse of some sort and have t_3 propagation. Perhaps each quartet becomes a cube with 8 corners, but I don't think anything new is really added here. An advanced topic.

In my MRS dream, you would get pictures like page 400 for each player in an MS lesion, ID the chemical from the dots, and watch how things move in time! The advantage is that you know pretty much what most of the players are. But maybe there are lots of ones that are not known, that appear only in vivo.

B. INADEQUATE

13.2 INADEQUATE (399). // invented by Ad Bax & friends in 1980

13.2.1 About ^{13}C isotopomers. (399). Another long section with lots of theory, similar to the COSY section above. The point of this method is to suppress strong peaks so you can see the small peaks you want to see. As an example, ethanol has two carbons, and the density of molecules with *both* C's being ^{13}C is very low. This is iso IV and is .012%. Only these special molecules will behave like the AX J-coupled system we have been studying. Page 402 shows that you get two strong peaks because the two carbons have different chemical shifts, and of course these peaks are from the 1% single-C-13 molecules. The double-peak "satellites" are there, but you cannot see them due to the huge peaks.

Page 403 top shows a real world example. For the molecule shown, there are 45 pairs of possible isotopomers with two C13's. In the "conventional spectrum" of figure (b) page 403, you cannot see any satellites. But somehow the bottom traces is supposed to show these satellites (but I don't know how to read this spectrum).

The reason you might do such a thing is that you can learn about molecular shape. One note on the picture: they ran this NMR experiment for 16 hours!

13.2.2 Pulse Sequence (403). It is shown on page 403 bottom and in fact the phases ϕ_1 and ϕ_2 and ϕ_3 will all be zero, so we just have standard pulses in the ^{13}C timeline as shown. The last phase gets cycled through four values. The last two pulses "abut". It seems clear to me that the middle part is a tuned spin echo sandwich. (tuning top page 404 says $\tau = 1/(2J)$.)The I program is to blast the H1 spins so they can't make any signals. Malcolm has never really explained how this decoupling works. I guess if you blast away, the H spins just rotate around the x-axis all the time and this makes any spin direction equally likely in an experiment, so it's coupling to other spins should wash out. I guess I could to run the propagator and look what happens, but I will accept this decoupling idea for now. So at least I understand the picture at the bottom of page 403: S = C13, I = H1. The prewash signal also helps, and this will be talked about in a later chapter. We really need to concentrate on this AX system aspect here. Note that C13 does have a spin-1/2 ground state which is good because that is the only AX J-coupled system we have looked at. This section then describes the cycling sequence.

Comment: If you "blast the $_1$H nuclear spins" as just mentioned, during the RF blasting pulse these spins will nutate in the rotating frame. Imagine this as one long $(\beta)_x$ pulse so the hydrogen nuclei just tumble around the x axis at the nutation rate which recall is $1/2\gamma B_{RF}$. Probably this is fast enough to wash out any interactions between these H spins the carbon spins you are looking at.

13.2.3 Theory of INADEQUATE (405). As discussed earlier, we can replace the sandwich as shown, so we must be in one of the two limiting cases mentioned in the appendix on this subject, but Levitt does not comment on this, presume OK. Notice that now you get an unusual $3\pi/2$ x-pulse on the left. $(\pi/2)_{-\mathbf{x}}$.

(1) Show that regular single-C13 spins are suppressed [p 405-407] , ie, those from most of the molecules having one C13 spin. If we were in TE, we would have $\rho = 1/2 + 1/2BI_{\mathbf{z}}$ as shown page 284. But our little pre-pulse applied to the H's somehow affects the C13's and we get instead that $\rho = 1/2 + 1/2\ \varepsilon_{\mathbf{NOE}}\ BI_{\mathbf{z}}$ where $\varepsilon_{\mathbf{NOE}}= 2$ (Nuclear Overhauser Enhancement effect). But all we need to look at is our $I_{\mathbf{z}}$ factor and watch what happens. I verified every step in this presentation on pp 406-407 and the conclusion is that as you cycle through the four phases, everything does cancel. You basically *select* $\phi_{\mathbf{4}}$ and $\phi_{\mathbf{dig}}$ (page 404 bottom) to make this happen.

Comment added: This page 406 picture applies to single-C_{13} molecules and this have just a single spin, so we are not talking AX systems here. If "only J couplings survive" in the middle, and there are no J couplings, then that is why the τ step does NOTHING in the "equivalent sequence shown as lower picture on p 405! Remember that one of our big goals here is to suppress these peaks, and this pulse sequence does the job!

(2) Show that the AX coupled spins survive [p 408-410]. Here I again verified things where shown, but I did not do 13.8 (I will do it below -- **DONE**). The result is that all four phases are additive for the J-coupled stuff, and so they survive the pulse sequence protocol. If you study each of the four single-quantum coefficients a, you get the alternating pattern shown in (13.10,11) and this means the spectrum is a pair of anti-phase doublets as shown on page 410 top (the β's go down). The claim is that you sort of see such things in the page 403 spectrum, but these things seem to be further split or distorted, no comments are given.

So the point is very clear: this fancy pulse protocol kills off the normal chemical shift single-spin lines in the spectrum, and only the rare J-coupled signals survive. The amplitude of the pulses is not small in the sense of proportional to J or anything like that! The amplitude is full bore, but it is cut down by the rarity of the double C13 isotopes. The role of J was to set our τ period so $\pi J\tau = \pi/2$. If J is weak, we just have to use a longer period. So in this sense, the J-coupling is as strong as anything else!

13.2.4 Coherence transfer pathways (410). We are getting more sophisticated now. The claim is that your can trace the flow of coherence type (like 0, +1, -2 etc) through the pulse sequence (thinking in terms of cycling as well). For example, in TE we know there are no off-diagonal ρ terms, so everything is zero-quantum at that point.

Now the claim is first that the $(\pi/2)_{\mathbf{x}}$ pulse converts all of the 0 to +1 and -1 "flow". Well, we know that this pulse takes $I_{\mathbf{z}}$ down to $-I_{\mathbf{y}}$ (really sum of 1 and 2), and each of these can be written as raising lowering and indeed, you have $\pm\alpha$ type matrix elements after this, so indeed, you have "converted" the 0 to equal amounts of +1 and -1 (several terms of each kind).

What does the $\pi_{\mathbf{x}}$ pulse do? He gives an example bottom page 410 which says roughly that this pulse makes changes like $-\alpha \rightarrow +\beta$, so this must swap the +1 and -1 sense whatever flows come in. It would perhaps swap the ++ type components as well, but there is no flow in those pipes at this point.

Comment added: $(\boldsymbol{\pi})_x$ $\Rightarrow \exp(-i\pi J_{\mathbf{x}})\ J_{\pm}\ \exp(+i\pi J_{\mathbf{x}}) = J_{\mp}$, a general fact, so this confirms what happens at the cross on page 410. If we had $I_1^+\ I_2^+$ coming in on the +2 line, it would go out on the -2

line as $I_1^+ I_2^+$, so in general $(\pi)_x$ "negates" whatever comes in of non-zero order, and passes the zero order freely.

Now what happens when $(\pi/2)_x$ acts on the +1 and -1 coherence flows? We saw halfway through page 408 between time 3 and time 4 that it tips down the z's so you get x-y products and these are the double stuff. So that explains the next part of the flow where, in the gap between the last two pulses, we have only double quantum flow. When you then apply the last pulse in the full sense of its cycling with various things changing in the cycle, you find that the "back end" of this pulse sequence acts as a **filter** and only lets the double coherence stuff through!

It is always the J-coupling rotation that "activates" the double quantum flow, and this is missing when you inject single chemical shift flow through our pathway. There is no double, so nothing gets through the final filter. Following comment clarifies this point.

Comment added: In other words, suppose we had J = 0 coming in. In that case, according to upper page 406 (applied to 2-spins) we would end up at point 3 with $\rho_a = I_{1y}+I_{2y}$ instead of $\rho_b = -2I_{1x}I_{2z} -2I_{1z}I_{2x}$. This is because the SES's τ is tuned for the specific molecular J value so we get some finite $\tau = 1/2J$. If you run zero-J signal through this tuned SES, the propagator for going 2 to 3 in the equivalent picture is unity! Now both the ρ's just quoted have ± 1 order, but in terms of my "ρ vectors" (see elsewhere), these are different ρ vectors which have the same "order vector" O. In other words, at point 3 we have $\boldsymbol{\rho}_a \neq \boldsymbol{\rho}_b$ but we have $\mathbf{O}_a = \mathbf{O}_b$ so on an order flow diagram you cannot see the difference. But there is a difference and it is this: when ρ_b comes into the last $(\pi/2)_x$ pulse, it is converted into ± 2 order, whereas when the ρ_a comes in, it is converted into 0 order, as indicated by the final result ρ_a(point 4) = $I_{1z} + I_{2z}$. So one point to keep in mind is that the heavy line flow through the last $(\pi/2)_x$ pulse in the page 410 figure (ending up with ± 2) is *specific* to the specific J-tuned signal coming in!!! For zero-J input, the picture would look the same up to point 3, but then you would see the $\pm$ 1 lines flow to the 0 line at time 4. Then since the phase-cycled last pulse acts as a 4-spaced filter, this flow is blocked!

The proof that the last stage is in fact a ± 2 coherence filter is shown in Appendix 17.10. This is a huge 26 page appendix, so I suspect this subject is of great interest to Malcolm. The subject of this appendix is "phase cycling". See notes elsewhere.

Question: Why do we say that the +2 (for example) is fed from *both* +1 and -1 in page 410 picture at point 4? I don't think this is obvious, and I will ask this question again when I solidify my "formalism" for what goes through a pulse.

Derivation of 13.8 , 13.9, and 13.11 on page 409. // referred to above; out of order

First, do 13.8: Let's take the [0] case of ρ_5 which is this:

$$- 2I_{1z} I_{2y} + (1 \leftrightarrow 2)$$ // verified

We can write $I_{1z} = (1/2) (I_1^{\alpha} - I_1^{\beta})$ projectors and $I_{2y} = (1/2i)[I_2^+ - I_2^-]$ (both from p 166) to get

$$- (I_1^{\alpha} - I_1^{\beta}) (1/2i)[I_2^+ - I_2^-] - (1/2i)[I_1^+ - I_1^-](I_2^{\alpha} - I_2^{\beta})$$ // case [0]

and this represents these eight single-quantum terms:

$\alpha+ \quad \alpha- \quad \beta+ \quad \beta- \qquad\qquad +\alpha \quad -\alpha \quad +\beta \quad -\beta$

If we focus on just the -β term as he suggests, then that term is this

$$- \quad (1/2i)\,(\; - \; I_1^-)\,(\; - I_2^{\beta}\,) = - \quad (1/2i)\;\; I_1^- I_2^{\beta} \;=\; + (i/2)\, I_1^- I_2^{\beta}$$

and we then obtain the i/2, just as he shows (for the actual matrix element of ρ).

Now lets try the [1] case. Start with

$$- 2I_{1x}\, I_{2z} + (1 \leftrightarrow 2)$$

We can write $I_{2z} = (1/2)\,(\, I_2^{\alpha} - I_2^{\beta}\,)$ projectors and $I_{1x} = (1/2)[\, I_1^{+} + I_1^{-}\,]$ (both from p 166) to get

$$= - (1/2)[\, I_1^{+} + I_1^{-}\,]\,(\, I_2^{\alpha} - I_2^{\beta}\,) \quad (1 \leftrightarrow 2)$$

but we can see the -β term is this

$$- (1/2)\, I_1^- (- I_2^{\beta}) = (1/2)\, I_1^- I_2^{\beta}$$

which verifies the second result in 13.8. The third term is minus the first, and the fourth minus the second, so we are all done.

Next, do 13.9 and 13.10. Now use $a = (2i)\,\rho \exp(-i\phi_{dig})$ on each term:

[0] gives $i/2 * 2i * \exp(-i0) = -1$
[1] gives $1/2*2i \; * \exp(-i3\pi/2) = 1/2*2i*i = i^2 = -1$

The next two cases have $\phi_{dig}\,\pi$ different from the above, so get extra -1. But that cancels the extra -1 we pick up in (13.8) in the last two terms, so both these cases give -1 as well. QED.

Finally do (13.11). Now having done all the above for the -β term, how are things different for the -α term? The projectors appear as $(\, I_1^{\alpha} - I_1^{\beta}\,)$, so we will always get a reverse sign relative to our previous four answers which were all -1, so these will all be +1, which agrees with the first in 13.11.

OK, go back to -β and ask "how will the β- term differ from the -β term" ? Looking above at the [0] case, we see that these two terms have the same sign. This is caused by the (1↔2) relationship of the second terms to the first terms, and this relationship holds for all four cases, so the answer is that -β is the same as β-, and that is what we see in 13.11, another -1. [but of course these have

Finally, we know the α- will be the same as the -α for the exact same (1↔2) reason, so we have now derived 13.11 as well.

Remember that the spectral peak location is determined by Ω^0_1 for -α and Ω^0_2 for α- since the - sign says where the quantum transition is located. The final FID period runs at expo of the corresponding energy and that controls where the peak is. This fully explains page 410 top.

So the upshot is that we get two pairs of anti-phase peaks. I have marked on page 403 where one such pair might be in the real-world sample spectrum, though polarity is reversed and there is other stuff going on so not clean.

The text on pages 411-412 just repeats things I have already said above, that you can take a coherence-blocking view of the whole thing and see why J=0 is blocked and J_{tuned} passes. Since you know that all four output phases are additive, you can just calculate one of them to get the final ρ result which is what appears at the bottom of page 411, and from which you see at once the pair of anti-phase peaks.

OK to here on review of INADEQUATE 1.27.08 and again on 1.29.08.

13.2.5 Two dimensional INADEQUATE.

Add t_1 gap between last two pulses, fine. The calculations which follow are very difficult for me to do, I cannot do them without errors it seems. Need a computer program to do them really.

---------- **Derivation of equations on page 414** ----------

(414). Derive the upper result: The acting H is the three z terms so we get the usual two rotations, but we act now on $2I_{1x}I_{2y} + 2I_{2x}I_{1y}$.

Let's just work on the first term and apply our three rotations to it as noted on page 374.

$$R_{1z}(\Omega_1 t)\, R_{2z}(\Omega_2 t)\, \exp(-i\phi\, [2\, I_{1z}I_{2z}]) \qquad \phi = \pi J t$$

Let's ponder this a bit. Any of these three rotations is going to make S and C term mixes so the result will balloon out quickly. Let's try the worst one first and see what it does. It turns out that it does nothing at all! The reason is this:

$$[\ 2\, I_{1z}I_{2z}\ ,\ 2I_{1x}I_{2y}\ ] = 0 \qquad \text{// from page 379 bottom!}$$

I did not realize this until I did all the math and found that nothing happens! So we can just forget the double z term, and we see that the result is not going therefore to depend on J.

So now let's take $2I_{1x}I_{2y} + 2I_{2x}I_{1y}$ and subject it to the other two rotations. But I think for this step, it is better to write this as on page 408 as -i $(I_{1+}I_{2+} - I_{1-}I_{2-})$. Then we shall use this sandwich rule

$$\exp(-\,i\,\theta\,J_z)\ J_\pm\ \exp(+\,i\,\theta\,J_z) = \exp(\mp i\theta)\, J_\pm$$

Now just apply both rotations at once to get, acting on $(I_{1+}I_{2+} - I_{1-}I_{2-})$,

$$= R_{1z}(\Omega_1 t)I_{1+}R_{1z}(-\Omega_1 t)\, R_{2z}(\Omega_2 t)I_{2+}R_{2z}(-\Omega_2 t)$$ - same with minus signs on the I's

$= \exp(-i\Omega_1 t)\exp(-i\Omega_2 t)\, I_{1+}I_{2+} - \exp(+i\Omega_1 t)\exp(+i\Omega_2 t)\, I_{1-}I_{2-}$

$= \exp(-i\alpha)\, I_{1+}I_{2+} - \exp(+i\alpha)\, I_{1-}I_{2-}$ $\quad \boldsymbol{\alpha = (\Omega_1+\Omega_2)t}$

$= (C\text{-}iS)\ I_{1+}I_{2+} - (C\text{-}iS)\, I_{1-}I_{2-}$

$= C\,(\, I_{1+}I_{2+} - I_{1-}I_{2}) - iS(\, I_{1+}I_{2+} + I_{1-}I_{2-})$

We know that

$I_{1+}I_{2+} = (I_{1x} + iI_{1y})\,(I_{2x} + iI_{2y}) = I_{1x}I_{2x} - I_{1y}I_{2y} + i\, I_{1y}I_{2x} + iI_{1x}I_{2y}$
$I_{1-}I_{2-} = (I_{1x} - iI_{1y})\,(I_{2x} - iI_{2y}) = I_{1x}I_{2x} - I_{1y}I_{2y} - i\, I_{1y}I_{2x} - iI_{1x}I_{2y}$

Therefore

$I_{1+}I_{2+} + I_{1-}I_{2-} = 2I_{1x}I_{2x} - 2I_{1y}I_{2y}$
$I_{1+}I_{2+} - I_{1-}I_{2-} = i\, 2I_{1y}I_{2x} + i2I_{1x}I_{2y}$

So our result is then

$C\,(\ i\, 2I_{1y}I_{2x} + i2I_{1x}I_{2y}) - iS(\ 2I_{1x}I_{2x} - 2I_{1y}I_{2y})$

Now apply the overall $-i = 1/i$ omitted above to get

$\rightarrow C\,(\, 2I_{1y}I_{2x} + 2I_{1x}I_{2y}) - S(\ 2I_{1x}I_{2x} - 2I_{1y}I_{2y})$ // result A

$= (-i)\ \{\ \exp(-i\alpha)\, I_{1+}I_{2+} - \exp(+i\alpha)\, I_{1-}I_{2-}\ \}$ // result A

and this does **agree exactly with Result A** quoted, so I now check that off. [I think this was a hard way to do it!]

Now apply $(\pi/2)_y$ which takes z to x and x to -z resulting in

$\rightarrow - C\,(\, 2I_{1y}I_{2z} + 2I_{1z}I_{2y}) - S(\ 2I_{1z}I_{2z} - 2I_{1y}I_{2y})$

which **agrees exactly with result B** so I red check that one off. // result B

Now go back to result A and do the next step a different way. We had

$= (-i)\ \{\ \exp(-i\alpha)\, I_{1+}I_{2+} - \exp(+i\alpha)\, I_{1-}I_{2-}\ \}$

Let's try doing $R_y(\pi/2)$ directly on this thing, omitting the (-i) for the moment:

$R_y(\pi/2)\, J_{\pm}\, R_y(-\pi/2) = \pm\, i\, J_y - J_z$ or $R_y(\pi/2)\, I_{1\pm}\, R_y(-\pi/2) = \pm\, i\, I_{1y} - I_{1z}$

// checked

This gives, acting on the result above

$$\exp(-i\alpha)\,[\,+\,i\,I_{1y}\,-\,I_{1z}]\;\;[\,+\,i\,I_{2y}\,-\,I_{2z}]\;\;-\;\;\exp(+i\alpha)[\,-\,i\,I_{1y}\,-\,I_{1z}]\;\;[\,-\,i\,I_{2y}\,-\,I_{2z}]$$

$$=\;\exp(-i\alpha)\;\{\;-\,I_{1y}\,I_{2y}+\;I_{1z}\,I_{2z}-i\,I_{1y}\,I_{2z}-\;i\;\;I_{1z}I_{2y}\;\}$$
$$-\;\exp(+i\alpha)\;\{-\,I_{1y}\,I_{2y}+\;I_{1z}\,I_{2z}+i\,I_{1y}\,I_{2z}+\;i\;\;I_{1z}I_{2y}\;\}$$

$$=\;(-\,I_{1y}\,I_{2y}+\;\;I_{1z}\,I_{2z})\;[\,\exp(-i\alpha)-\exp(+i\alpha)]\;\;-i(\,+\,I_{1y}\,I_{2z}\;+\;\;I_{1z}\,I_{2y})\;[\,\exp(-i\alpha)+\exp(+i\alpha)]$$

$$=\text{-}2i\,\sin\alpha\;(-\,I_{1y}\,I_{2y}+\;\;I_{1z}\,I_{2z})\;\text{-}\;i\;2\cos\alpha\;(\,+\,I_{1y}\,I_{2z}\;+\;\;I_{1z}\,I_{2y})$$

Now add back our overall (-i) = 1/i factor from -i ($I_{1+}I_{2+} - I_{1-}I_{2-}$) and we get

$$=\text{-}2\,\sin\alpha\;(-\,I_{1y}\,I_{2y}+\;\;I_{1z}\,I_{2z})\;\text{-}\;2\cos\alpha\;(\,+\,I_{1y}\,I_{2z}\;+\;\;I_{1z}\,I_{2y})\qquad\qquad\text{// second term OK}$$

$$=\{\;\text{-}\;(\;2I_{1z}\,I_{2z}\;\text{-}\;\;2I_{1y}\,I_{2y})\sin\alpha\;\;-\;(\;\;2I_{1z}\,I_{2y}\;+2\;I_{1y}\,I_{2z})\cos\alpha\;\;\}$$

which finally agrees with Result B. This calculation took me about 2 solid hours or more! [I think there are easier ways, but I won't dwell on it right now.]

Calculate the sine expression

Now we keep only the order -1 portion of the above and we have

$$\rho^{\mathbf{cos}} =\;-\,(\;\;2I_{1z}\,I_{2y}\;+\,2I_{1y}\,I_{2z})\cos\alpha$$

Now we want to do the "sine term". What is different here is the entire sequence from 1 to 4, because now all the phases have been rotated, and ψ = - 45 degrees = $-\pi/4$. This is a lab rotation, so this should be the same as an observer rotation by +45 degrees around z. Looking back at "confusion...doc" if I think of ρ as an operator at time 4, if we rotate the lab by = -45, then the operator does this:

$$\rho' = R_z(-45)\rho R_z(-45)^{-1}$$

So let's apply this on our result at time 4 which was this:

$$(\text{-}i)\;\{\;\exp(-i\alpha)\;I_{1+}I_{2+}\;-\exp(+i\alpha)\;I_{1-}I_{2-}\;\}$$

Now go look up the relevant rule,

$$\exp(-\,i\,\theta\,J_z)\;\;J_{\pm}\;\exp(+\,i\,\theta\,J_z)\;\;=\exp(\mp i\theta)\;J_{\pm}$$

$\exp(-i\theta I_{1z})\ I_{1\pm} \exp(+i\theta I_{1z}) = \exp(\mp i\theta)\ I_{1\pm} = \exp(\pm i\pi/4)\ I_{1\pm}$

Now set $\theta = -\pi/4$ and get $= \exp(\pm i\pi/4)\ I_{1\pm}$, as shown above.

Then we get

$$(-i)\ \{\ \exp(-i\alpha)\exp(+i\pi/2)I_{1+}I_{2+} - \exp(+i\alpha)\exp(-i\pi/2)\ I_{1-}I_{2-}\ \}$$

$$= (-i)\ \{\ \exp(-i[\alpha-\pi/2])\ I_{1+}I_{2+} - \exp(+i[\alpha-\pi/2])I_{1-}I_{2-}\ \}$$

$$= (-i)\ \{\ \exp(-i\beta)\ I_{1+}I_{2+} - \exp(+i\beta)\ I_{1-}I_{2-}\ \} \qquad \beta = \alpha - \pi/2$$

So we get our same result as before but with β in place of α. But

$$\cos\beta = \cos(\alpha - \pi/2) = \cos(\pi/2-\alpha) = +\sin\alpha$$

$$\sin\beta = \sin(\alpha - \pi/2) = -\sin(\pi/2-\alpha) = -\cos\alpha$$

Therefore, the Result A is "as was for cos" but with the above swap. This is true also of Result B then. For the -1 part, it is the $\cos\alpha \rightarrow +\sin\alpha$ that is relevant and this yields result C, another red check..

What does the spectrum look like? The result we have obtained here is the same as the simple inadequate result shown at the bottom of page 411 *except* for the overall $\cos\alpha$ or $\sin\alpha$ multiplier. This means that the Ω_2 spectrum is exactly the same as in 1D inadequate, but we have forced a line in Ω_1 at the sum frequency! And of course we meet the States criteria, so we get the nice result on page 415.

The sum frequency is associated with a ++ double quantum activity (propagation in this case during the gap). The t_1 variable let's you "see" what kind of propagation is going on during that gap, even though it is not observable from the FID. It might be observable if you tune the receiver for it and have an earlier FID, etc.

I agree with the look of the spectrum on page 416, but his nice picture is not drawn to scale, a minor point.

Example: in sucrose, you have 10 C-C bonds and each one will show up (I presume) as four dots in a row. The picture seems to show 9 of these sets, maybe one is degenerate. Malcolm was involved in the 1981 paper that did this spectrum, a mere 27 years ago!

Exercise: Compare DQF-COSY to 2D INADEQUATE.

Both provide **States** clean peaks. From a pair of spins, COSY gives 4 quartets of peaks meaning **16 peaks**. INADEQUATE gives **four peaks** on a horizontal line. From either you could measure both chemical splitting and the J value from the peak locations. INADEQUATE has the **big advantage** of blocking the single-spin main lines! COSY first done by Jan Janeer in 1971, not published I think until 1976 by student Ernst. INADEQUATE was Ad Bax in 1980. I think COSY is used with proton spins

which are always present, while INADEQUATE is used with ^{13}C which makes it have low sensitivity due to isotope fractions. But why this distinction? Web is not telling me quickly, but I see there is a lot of stuff out there on these methods! These are both AX homonuclear methods. You really can ID chemicals with these methods.

C. INEPT

13.3 INEPT

13.3.1 The sensitivity of nuclear isotopes. (416) The point here is that large γ spins give much stronger NMR signals for many reasons, and roughly NMR S/n ~ $\gamma^{2.5}$. Comparison of N^{15} and H^{1} γ shows a factor of 10, so get 300x increase in S/N, all other things equal. This fact provides a motivation for why we want to do "magnetization transfers" when dealing with small-γ spins, as explained below.

13.3.2 INEPT pulse sequence. (418).

This our first *hetero*nuclear AX experiment. Since the Larmors are far apart, you need two transmitter systems, so we always have I and S orchestra score lines. In 2D INADEQUATE we had two scores, but we were just blasting the protons to prevent them from splitting our ^{13}C nice peaks, see page 413 score.

Our INEPT scores play different music. The proton score is the I line which is the high-γ species. For the protons, we do a knockdown, then a SES, then a final FID-maker pulse. For the ^{15}N we omit the first knockdown pulse, have the same SES (perhaps tuned to *its* Larmor, does not say), then a different FID type closure pulse. We look at the S FID.

Here is a quick summary of what happens before we comment on details. You start with your TE which includes both spin types which he writes here as I_z and S_z rather than I_1 and I_2 -- perhaps this is the hetero convention. To trace the ρ matrix, when there are simultaneous score events, we do either one first, then the other one, because they act on separate spin operators. Maybe it would be better to call the spins I_I and I_S. When we start in TE, we have terms $B_I I_{Iz}$ and $B_S I_{Sz}$ where B = Boltzmann and contains the γ factor for that spin type. At the end, we get terms like $B_I I_{Iz} I_{Sy}$ and $B_S I_{Sy}$. These just arise normally as you act on your spins with the various selected pulse sequence elements, see pages 420-421. The point is that you have "transferred" the large B_I Boltzmann factor over to the S species transverse spin, so you will now get a large NMR signal at the S = ^{15}N frequency. This is called **"magnetization transfer"**.

Now some comments. What would a *simple* experiment be with this system? Suppose you blasted on the H and did a single knockdown on the N ? You would get $B_S I_{Sy}$ and your signal would have the strength of B_S. This is shown as plot (b) on page 422. You see two tiny positive peaks in this (b), and nothing at all in (a) [same but no proton blasting] for this amount of data collection.

Now for INEPT we get the terms $B_I I_{Iz} I_{Sy}$ and $B_S I_{Sy}$ as just noted. These appear as on page 421: a very small positive set of two peaks, then a large antiphase peak pair, as expected. These appear in the experiment additively so you get the "total" shown. And you see this in plot (c) page 422.

Now, what would have happened if you just did an H knockdown? Get $B_I I_{Iy}$ and see this signal strongly. Suppose we did COSY or INAD on the I channel? Does this not work for some reason because we are hetero? Web says that hetero-COSY is done all the time.

I guess the point here is that if you *want* to do a FID at the ^{15}N frequency, you will get a 10x stronger result by doing this INEPT "magnetization transfer" method. One reason might be that there is a lot of competing signal on the I channel (I would imagine) from any normal compound.

I wonder what is happening graphically here? You can see that the J-coupling propagation phase between times 2 and 3 is what causes the actual "transfer". It converts I_{1y} to $2I_{1x}I_{2z}$ and this is where the mix occurs. This is just what a SES does for J-coupling. It is $2I_{1z}I_{2z}$ in the Ham that causes it. The final steps just change the result to $B_1I_{1z}I_{2y}$ so it is the I_2 magnetization that has the big B_1 on it!

The Decoupling Story

The question here is this: why is decoupling (my "blasting") like setting J = 0 during the FID. This is a very tricky subject. When we do COSY or anything where we have J-coupling, we run into this kind of transformation:

$$\exp(-i\,\phi\,2I_{1z}I_{2z})\;\; I_{1x}\;\exp(+i\,\phi\,2I_{1z}I_{2z}) \;\;= I_{1x}\,C_\phi \;\; + \;\; 2I_{1y}I_{2z}\,S_\phi \qquad\qquad [\;\phi = \pi J\tau\;]$$

and this is operative during a free-propagation. This is what causes C_ϕ type factors to appear in the density matrix. The exponentials arise because, when Ham = $\phi\,2I_{1z}I_{2z}$ is time-independent, we can just expo the Ham times t to find out how states and operators move.

Now, suppose 1 and 2 are hetero spins and we do a decoupling blast on I_1. We know that decoupling will cause I_1' to rotate at its nutation frequency and at any time, we get an effective I_{1z}(effective) = $I_{1z}\cos(\omega_{nut}t)$, where we don't care about phase. This is the same as imagining that we have $J_{eff} = J\cos(\omega_{nut}t)$. So if we have blasting running on the I_1 channel, we have J_{eff} varying in this way.

This means our Ham is now time-dependent, so you can no longer do the expo Ham trick! This is the first main point here. So that above transformation does not even apply during our "gap". This is a good thing, because if we time-average the RHS of the above expression, we get 0, but the answer we are looking for is that the average of the RHS should be just I_{1x}.

In Chapter 7 we saw lots of examples of how we formed effective Hamiltonians by averaging them over motions. This was first done on page 185. The idea is that we have a lot of spin systems and the Ham is the sum of these all, and we can then form an effective average Ham. In the sum, things cancel out between different systems, so that is how we can justify it.

But here we are talking about a single spin system S and we are blasting the I partner of this J-coupled pair of spins. Somehow we want to argue that we are allowed to use $\langle H'\rangle = 2\pi J\langle I_{1z}\rangle I_{2z} = 0$. So this is *different* from the motional averages we do earlier in the book.

Here is one argument. We can write a formal solution of the TD SE as follows

$$\psi(t) = \exp\left[\,-i\int_0^t H(t')dt'\,\right]\psi(0)$$

Suppose we can write $H(t) = H_0 + H'(t)$ where $H' \ll H_0$. Then we can examine the solutions $\psi(t)$ of our unperturbed H_0 and we can determine the general time constant of the solutions. Suppose $\psi(t)$ is basically unchanged during any period Δt = 1 msec in these solutions, so that we would say $\psi(t)$ was slowly

varying relative to a timescale of 1 msec. Now, suppose H'(t) varies much faster than this, perhaps it has a period of 10^{-15} seconds. Rewriting the above we have

$$\psi(t) = \exp[\, -iH_0\, t \quad -i\int_0^t H'(t')dt'\,]\ \psi(0)$$

and we now make an approximation: if H'(t) varies in some periodic manner which is much faster than our time constant of the general solutions, then we make this replacement

$$\int_0^t H'(t)dt \;=\; t * [\ 1/t \int_0^t H'(t')dt'\ ] = t * \langle H'(t)\rangle$$

which is valid really only for times t long compared to the period of H'. But if we are willing to not think about $\psi(t)$ for $t < 10^{-15}$ sec, then our approximation is very reasonable. If our original solution had done something significant in this small timeframe, then we would have to reconsider, but we said its time constant was on the order of msec. So in this manner we end up with our approximation,

$$\psi(t) = \exp[\, -i(\, H_0 + \langle H'(t)\rangle)\, t]\ \psi(0)$$

So in our decoupling example, we have $H' = 2\pi J \cos(\omega_{nut} t)\ I_{1z}I_{2z}$ and $\langle H'\rangle = 0$. This is the same as saying that J=0. Now let's think about this using our COSY calculation, but let I_1 and I_2 be hetero spins. After the first pulse we are in free propagation during the t_1 interval. At the end of (or during) this interval we have this

$$\rho = \; -I_{1y}C_\phi C_{\theta 1} \; + \; 2I_{1x}I_{2z}\, S_\phi C_{\theta 1} \; + I_{1x}\, C_\phi\, S_{\theta 1} + 2I_{1y}I_{2z}\, S_\phi\, S_{\theta 1} \; + (1\leftrightarrow 2)$$

and after the second pulse (bringing us to start of FID) we have

$$\rho = -\, I_{1z}C_\phi C_{\theta 1} \; - \; 2I_{1x}I_{2y}\, S_\phi C_{\theta 1} \; + I_{1x}\, C_\phi\, S_{\theta 1} - 2I_{1z}I_{2y}\, S_\phi\, S_{\theta 1} \quad + (1\leftrightarrow 2)$$

Now $\phi = \pi J t_1$. The mechanism that causes ϕ to even appear here is the free propagation during t_1 when we have that $\pi J 2 I_{1z}I_{2z}$ Ham rotation operative. If J=0, then we set $\phi = 0$ in the above formulas, which causes $C_\phi = 1$ and $S_\phi = 0$. <u>This is a very different result from saying that $\langle C_\phi \rangle = 0$!</u> This latter approach is wrong because the above ρ expressions are not valid for time-dependent H.

It is useful to watch this after the products are expanded. For example, at FID start in COSY we have

$$\rho \;= I_{1x}(S_{\theta 1-\phi} + S_{\theta 1+\phi})/2 \; -I_{1z}I_{2y}\,(C_{\theta 1-\phi} - C_{\theta 1+\phi}) \; + \; (1\leftrightarrow 2) \qquad \text{// agrees (13.5) + (13.4)}$$

The first grouping is an in-phase pair of peaks. If we set J = 0 so $\phi = 0$, the two sine terms become equal and we in effect *add the heights of the two peaks* before we turned off J. Also, the two peaks move from their previous values $\theta_1 \pm \phi$ to a single double-height peak at θ_1.

In the cosine term we have anti-phase peaks. If we do ϕ=0 here, it kills the entire term. Here you say that the two anti-phase peaks meet halfway in the middle and cancel each other.

13.3.3. Refocussed INEPT (421) -- aka "enhanced INEPT". If we simply add another SES onto each channel, and track what this does to ρ, we end up with ρ as on top of page 424. We now have $B_S I_{Iz} I_{Sy}$ and $B_I I_{Sy}$, whereas before we had $B_I I_{Iz} I_{Sy}$ and $B_S I_{Sy}$. We have swapped the B's. Now the strong peaks are in-phase, not antiphase, so now when we decouple the I channel and set J = 0, the two peaks "add" in the sense described above, rather than cancel as they do if you don't "refocus" with that last SES. Page 424 shows how the pieces now add together, and some spectra appear at the bottom. Lower shows what happens if you *don't* put on that final SES pulse and decouple, while upper shows if you do the enhancement with the final SES.

So, page 423 shows that we are moving along in our pulse sequence complexities.

D. BICELLES

13.4 AX Systems in Weakly Oriented Liquids.

13.4.1 Angular Information (425). The idea here is that there is an "NMR J-coupling AX way" to discover (ferret out, tease out, elicit) the *orientation* of an AX chemical bond relative to the molecule in which it lies. The method works if the molecule in question has some asymmetry (not spherically symmetric) as suggested by the page 425 figure where it is an ellipsoid. The goal is to find the angle θ_{IS} of a bond relative to the molecular axis. As a side note, author says NOESY and ROESY are going to give us bond *length*, so here we are going to get bond *angle*.

13.4.2 Spin Hamiltonian (426). We now use many earlier results which were quoted in Chap 7 for anisotropic liquids. Everything has an average over ellipse axis orientation angle θ. Chemical shifts are the δ_{zz} shift tensor averages. The $I_z S_z$ term now picks up *both* the DD coupling *and* the J-coupling, so that is a first. Up to now we have ignored the DD coupling because the $\cos^2\theta$-1 averages to 0 in isotropics which we have been dealing with up to now. So far, we are just "setting up" for what is to come.

13.4.3 Bicelles. We are going to use a "buffer solution" containing these things because they are going to cause a certain amount of orientation of our ellipsoid molecules which would otherwise be randomly oriented causing d_{IS}= 0 (isotropic). [At first I confused the ellipsoids with the little bicelle Oreo cookie things.] The bicelles are a "media" which "generates a weakly oriented liquid solvent". These things are a special case of micelles (spheres) where energy seems to be lowered by having the very long tails mingle, since they are both hydrophobic. This must make the cookie shape be stable. Here is a web clip: (references 15 and 16 are 1988 and 1994)

1.2. Bicelles

Sanders and Prestegard introduced the bicelle for solid-state NMR studies of membrane-associated biomolecules [15,16]. Bicelles are discoidal lipid aggregates composed of long-chain phospholipid and either detergent or short-chain phospholipid. As shown in Fig. 1, the distinguishing structural feature of a bicelle is a central planar bilayer formed by the long-chain phospholipid, surrounded by a rim of short-chain phospholipid or detergent that shields the long-chain lipid tails from water. Bicelle size is controlled both by the ratio of long-chain:short-chain phospholipid (q) and the total phospholipid concentration (c_L). Dimyristoyl phosphatidylcholine (DMPC) is often used as the long-chain phospholipid component and it can be doped with phospholipids that have identical chain lengths but different headgroups (e.g., dimyristoyl phosphatidylserine (DMPS) or dimyristoyl phosphatidylglycerol) to alter the charge characteristics of the interface and provide

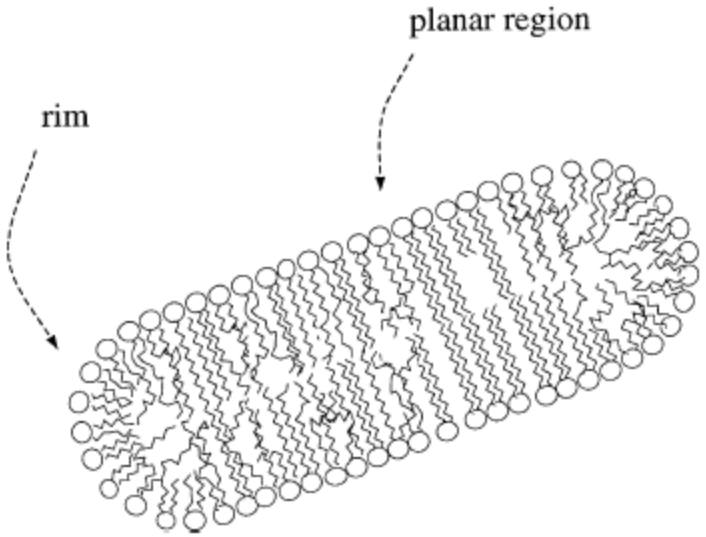


Fig. 1. Schematic representation of a bicelle showing the planar region, composed of long-chain phospholipid, surrounded by a rim of short-chain phospholipid.

The main point here is that, for reasons not given, the disks tend to line up parallel to the NMR B field as suggested in the picture top page 428. Then as they bang into each other, they tend to "stack" picking out a director direction as shown same place, so a little like a ferromagnetic domain alignment where the domain is the entire NMR sample. This in turn tends to line up the ellipsoid molecules, which is the main point, as suggested lower picture page 428. Orientation effect is very small 1 in 1000. The effect is that the ellipse molecules *tend* to have their short axes parallel to the director. The long axes tend to lie in a plane parallel to the bicelle domain plane.

13.4.4 Doublet Splittings (429). So, we have induced an anisotropic orientation for our ellipsoids, that was the goal of the bicelle solution. This causes all chemical shifts to change as per page 426, but more importantly, it turns *on* the formerly-zero residual dipolar interaction d which causes a change in the J-coupling splittings! The J coupling itself stays the same because it is not a function of orientation to the B field, whereas the DD coupling *is*. From (13.16), if the direction θ_{IS}=0 is favored in the average, d will be larger in absolute value. For our usual examples, however, b < 0 so alignment in effect reduces the J

coupling term causing a reduction in the splitting as shown top page 430. Malcolm gives a nice example of the angular average if the planar alignment of the long axes with the chip plane were perfect. In this case, the angular average has no sinθ term and the average becomes 1/4 instead of 0. So the upshot here is this: if you see the splitting get reduced, that says the AX chemical bond tends to point either along ellipsoidal axis or against it, since cos0 = cosπ = 1. Conversely, if the splitting has the other sign, you conclude that θ_{IS} is more tending to being perpendicular to the ellisoidal major axis. So we are getting here a tiny hint of the general direction of the bond, but only one component of this direction. He gives another average for this case and gets -1/8, I could do this if I wanted but let's not. The amount of splitting shift then tells you the z-component of a bond axis. Of course there could be many such bonds in your molecule, but they would be a different chemical shifts! The example here are C^{13}-H bonds.

So think about this. You have a molecule of some sort that is not spherical in shape (few molecules would be spherical, maybe methane or ammonia). You get an NMR signal from a single isolated bond in this molecule, and from the shift you perhaps know it is a C-H bond somewhere. Maybe from the amount of chemical shift you know which bond you are talking about. Then the fine detail noted here tells you to what extent that bond is oriented along or perp to the molecular long axis. That is a good trick considering you are not looking at the bond with your super atomic microscope. Perhaps on a spectral plot you study this for lots of bonds, and maybe you can reconstruct the molecular shape from this information.

13.4.5 Why not try for stronger orientation (431). The claim is that if you did this, then dipole couplings all become stronger and the spectrum becomes a total mess of overlapping junk. Of course it makes you wonder about some 2D method to pull things apart nevertheless.

Comment: so this is our first example of how orientation of a bond within its molecule can be studied. The signal is weak for C-H because you have to have a C^{13} for the C. But it is a 1D Spectrum, so don't need 2D time to run a test.

Exercises:

13.1 concerns HMQC (hetero multi quantum coherence, involves magnetization transfer like INEPT). This is a 2D States thing like COSY or INEPT, but pulses are different and I don't know what the spectrum would look like in our prototype example, but if I did the problem I would know.

13.2 is DQF-COSY and I have done all this in my Appendix 17_10 notes doc. The result is clean peaks for the diagonal we well as the off-diagonal quartets.