** This examination is open book, but is to be worked on ***independently***. You may not discuss or otherwise communicate *any* aspect of the exam with *anyone* other than C. Martin. This includes any discussions with anyone after you are done with the exam, but before the exam's due date and time. This is ***very important***.

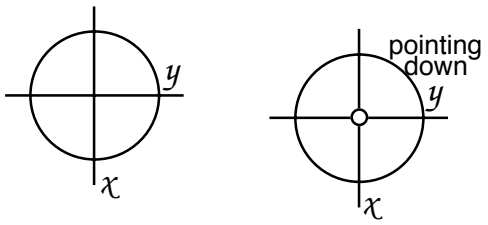
Due in class, 9:30am, Thursday, April 4

Show your work for full credit. Be concise, but complete.
Avoid long rambling answers which indicate that you don't really understand the question.

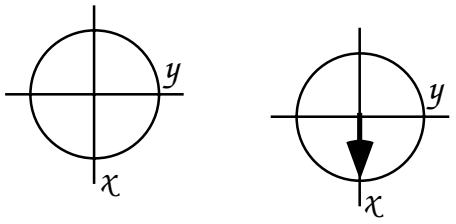
1. (5 points) Show, on the laboratory frame spin vector diagrams below, what will happen for each pulse sequence (ie. what is the state of the single magnetization vector precessing at ω_o immediately after the sequence?).

   Note: $\tau_D = \frac{\pi}{2\ \omega_o}$ The applied field lies along the z-axis, as usual.

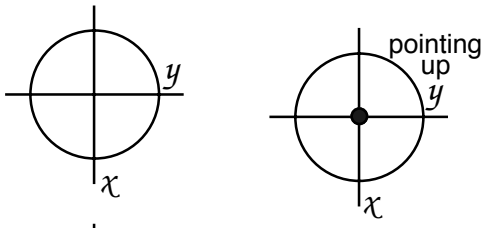
a) - $90°_x$ - $90°_x$ -



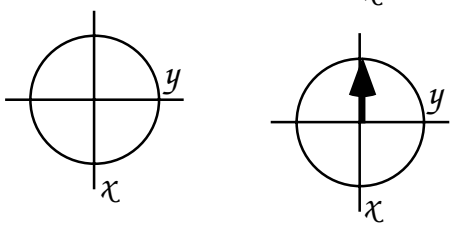
b) - $90°_x$ - τ_D - $90°_x$ -



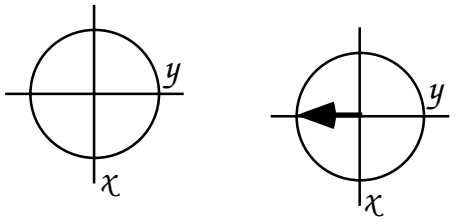
c) - $90°_x$ - $2\tau_D$ - $90°_x$ -



d) - $90°_x$ - τ_D - $180°_y$ -



e) - $90°_x$ - $2\tau_D$ - $180°_y$ -



2. (5 points) In a ^{1}H NMR experiment with an RF-generated pulse H_{xy} = 0.5 Gauss, for what length of time should one apply the RF field to achieve a 90° pulse? a 180° pulse? How long would one leave on the power to achieve 90° and 180° pulses for ^{13}C?

From before we know that $\theta = \gamma H_{xy} \Delta t$. Therefore $\Delta t = \dfrac{\theta}{\gamma H_{xy}}$

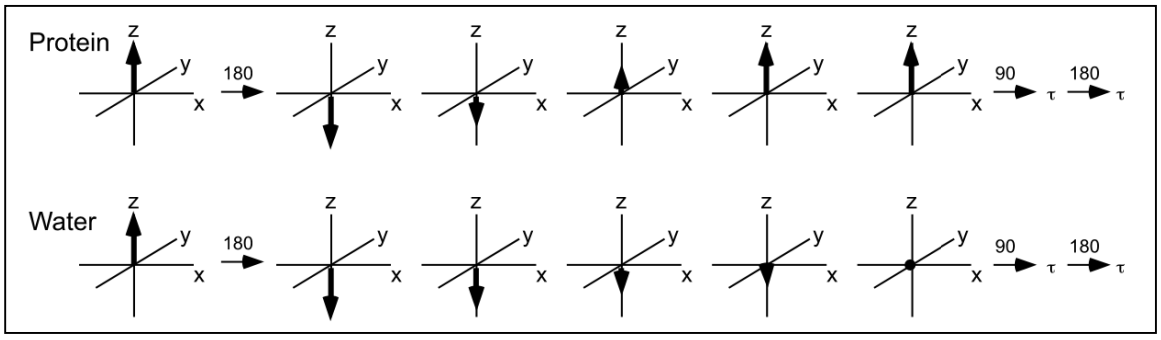
For 1H: $\Delta t(90°) = \dfrac{\pi/2}{26753 \text{ rad G-1 s-1} \quad 0.5 \text{ Gauss}} = 0.12$ msec $\Delta t(180°) = 0.23$ msec

For ^{13}C: $\Delta t(90°) = \dfrac{\pi/2}{6728 \text{ rad G}^{-1} \text{ s}^{-1} \quad 0.5 \text{ Gauss}} = 0.47$ msec $\Delta t(180°) = 0.93$ msec

3. (10 points) In protein NMR, even when the experiment is carried out in D_2O, there is always a very large signal due to water protons. If this signal is too large, it overwhelms the rest of the spectrum.

Fortunately, one can use pulse sequences to exploit relaxation effects to remove most of the water signal. Noting that water protons typically relax (T_1) much more slowly than protein protons, explain how the sequence <180-τ_D-90-τ-180-τ-acquire> can remove the signal due to water protons, leaving only the protein signals. How would you adjust the delay τ_D to optimize this effect? Think about what is happening to the bulk magnetization vectors of the two classes of protons.

The 180° pulse orients all spins along the negative z axis. Relatively quickly, the spins from the protein re-equilibrate to Boltzmann values (its magnetization vector returns to the positive z axis. In the meantime, the magnetization vector for water is slowly making its way back to the positive z axis. If we wait the right amount of time, the bulk magnetization from water will be half-way along, in other words, it will be 0! If at this point we start a regular <90-τ-180-τ-acquire> pulse sequence, we will get a regular spectrum from the protein, but *nothing* from the water. Cute.



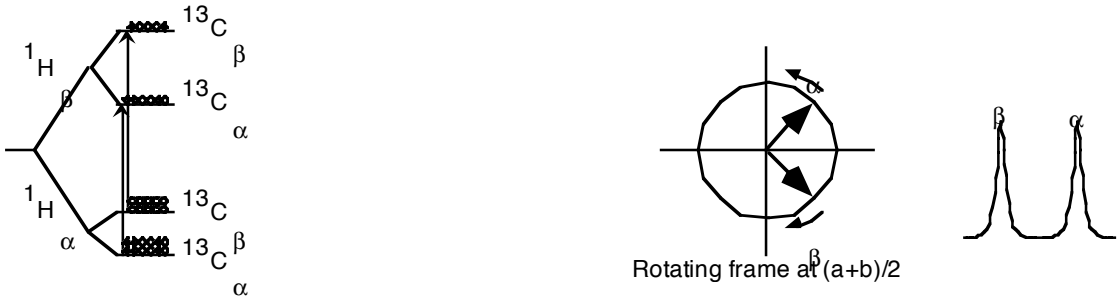
4. (20 points) The energy levels for a singlet ^{1}H split by a singlet ^{13}C are shown at right. The difference in populations between the levels is illustrated qualitatively. For each of the following situations (a-d), draw:

   1) Pictures illustrating the qualitative population distribution among the four energy levels immediately after the treatment indicated.

   2) A view, in the rotating xy frame, of the magnetization vectors for each ^{1}H, just after a 90°-acquire sequence, which is preceded by the indicated treatment

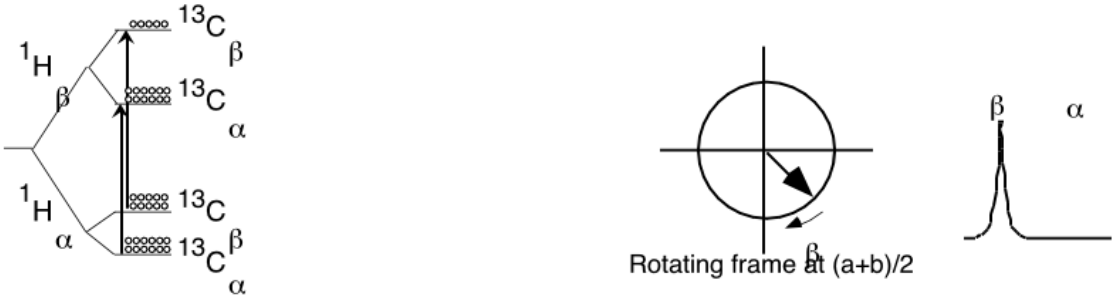
   3) The qualitative ^{1}H NMR spectrum you would expect to see, including relative intensities.

   x) (example) No pre-treatment.



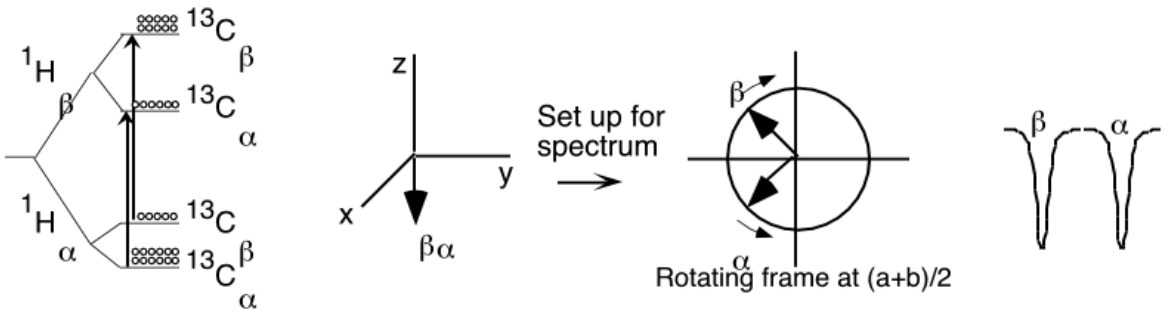
First, before any of the treatments below, we have the energy levels at their Boltzmann levels. To collect an NMR spectrum, we do our simple 90° pulse, followed by collection of the FID, to produce the spectrum shown at right.

a) Selective saturation (decoupling) of transition a (only).



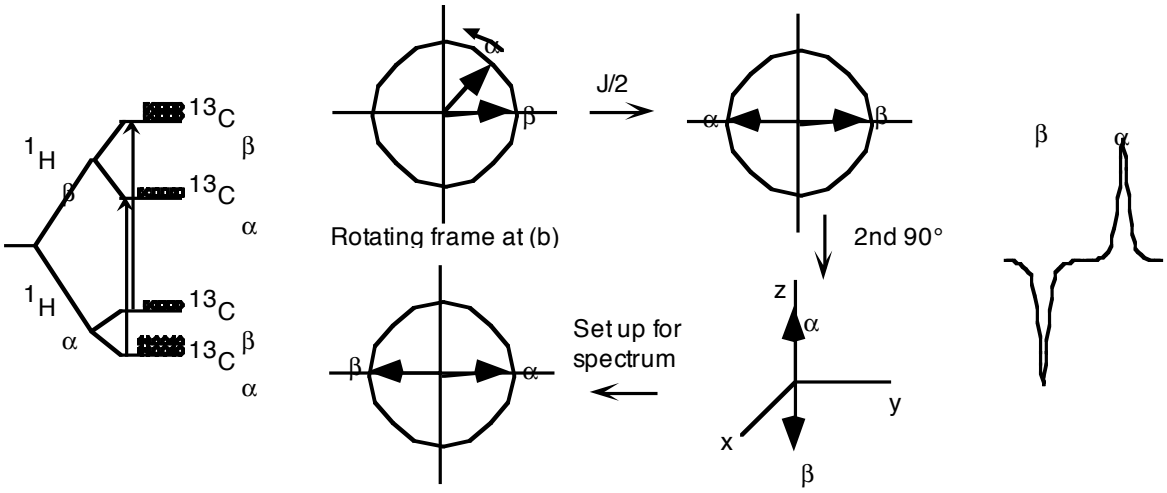
In this case, the populations of the levels connected by 'b' are made equal. Consequently, when we start our NMR experiment, the spins in those levels have no net bulk magnetization. We see nothing rotated into the xy-plane for those spins. We then see no NMR signal for that transition (in the old "electronic" way of looking at things - transitions are induced equally up and down, so there is no net absorption of energy in the classical scanning NMR experiment.

b) A very short 180° pulse along x (with no subsequent delay).



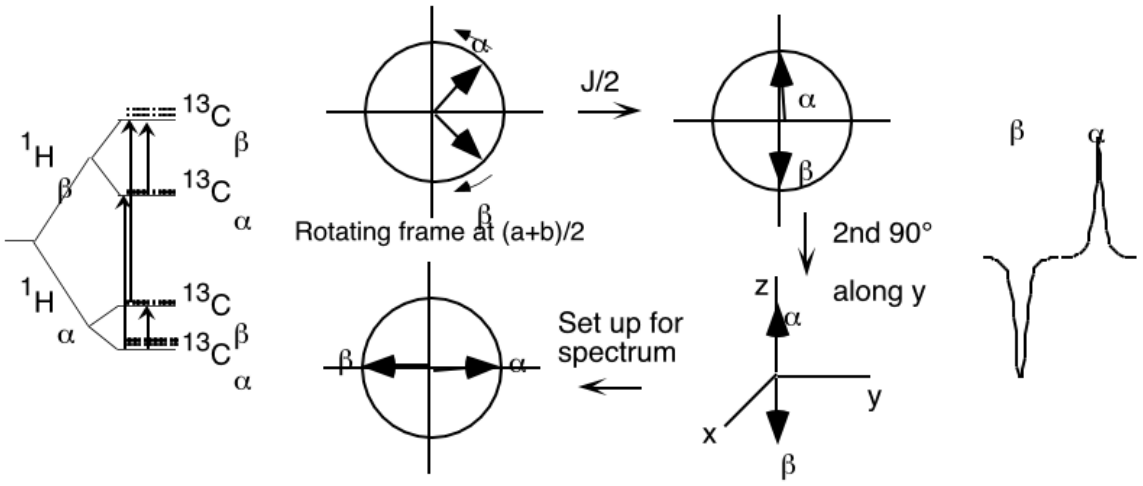
The prior execution of a broad frequency 180° pulse, inverts the z-magnetization. Thus, when we go to take our spectrum the spins get flipped to the negative y-axis. Their phasing is thereby 180° out from our normal spectra - this manifests itself as an inverted NMR spectrum. Again viewing things from the old way, the excited state is preferentially populated, such that application of an RF field *induces* emissive transitions.

c) Immediate pretreatment of the system with a pulse sequence of: 90°(x) - τ - 90°(x) with a relatively long pulse centered at ν = b, where $1/\tau = 2J_{^{13}C-^{1}H}$. Show the rotating frame at ν = b.



Since we are now in a different reference frame, α appears to move at twice its previous rate and β does not move at all. After the first 90° pulse, α precesses to exactly opposite β, such that the second 90° pulse brings it back to its original position along z, while β is inverted (its population is inverted). Now when we go to collect our spectrum , one spin system is inverted (as above), but the other is normal. The spectrum shows β with an inverted transition and α with a normal one.

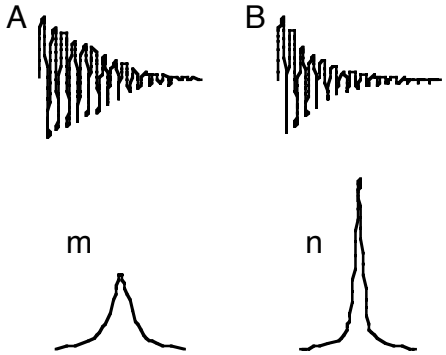
d) Immediate pretreatment of the system with a pulse sequence of: 90°(x) - τ - 90°(y) with a relatively long pulse centered at frequency (a+b)/2, where $1/\tau = 2J_{^{13}C-^{1}H}$. Show the rotating frame at (a+b)/2.



In this case, we return to the rotating reference frame of the chemical shift. This time, in the same time period, the two spins each execute a 90° rotation. Now a 90° pulse along the *y*-axis achieves a selective spin inversion.

5. Answer the following questions:

a) (5 points) in the figure at right, match the FID's with the resulting NMR spectral peaks.



A == n B == m

b) (5 points) in the application of post-collection processing of the FID, one can distort the FID (as above) to either increase or decrease the linewidth of the resulting NMR peak. What happens to the spectral noise associated with the resulting peak? Assuming that A and B are manipulations of the same original FID, which spectrum at right (m or n) will show higher signal-to-noise ratios (ie. less noise). The pictures, are ideal of course, and show no noise.

In A, later time (lower signal-to-noise) data is amplified. Thus there will be more noise in the spectrum that results from the FID

In B, later time (lower signal-to-noise) data is dampened (decreased). Thus there will be less noise in both.

6. (50 points) You have been given an unknown pentapeptide. On the following pages are COSY and NOESY spectra taken in partial D_2O. Amino acid analysis shows that the peptide contains one residue each of Asp, Ile, Leu, Ser, and Thr.

   a) (20 points) Assign the signals in each spectrum to individual amino acids

      On each spectrum, circle each cross peak group and write its assignment (eg. circle a multiplet and label it Asp-CαH in one direction and CβH in another).

   b) (20 points) Determine the sequence of the peptide, to the extent that you can. Comment on any amibuities.

   NH_2- Ser-Asp-Thr-Ile-Leu-COOH

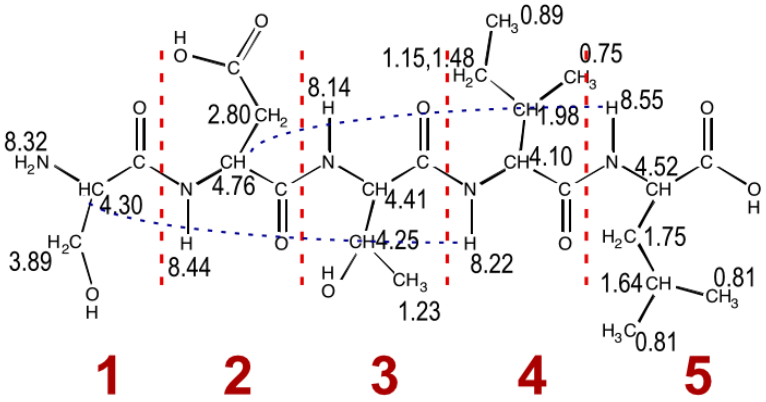
   c) (5 points) Some protons do not show up in any spectra. In your peptide which protons fall into which of these classes and why?

   In the presented spectra, the Ser NH2 and Leu COOH protons don't show up. They are in rapid exchange with D_2O, as is the Asp carboxylate proton. Finally, the Thr OH proton does not show up. This latter result is perhaps unrealistic, under conditions where amide protons are observed.

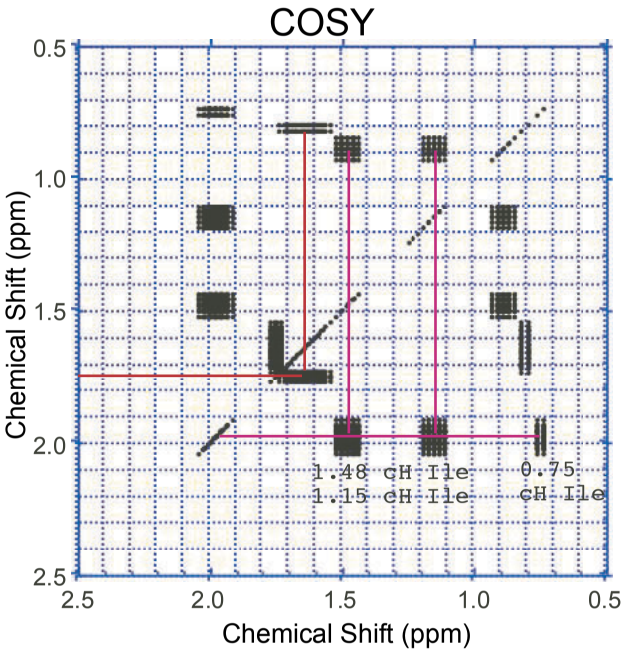
   d) (5 points) What new cross peaks might you expect if this short peptide formed a stable α-helix (if for example, it were run in deuterated trifluoroethanol which "forces" a peptide into an α-helix)? Draw them in on the appropriate spectrum.

In the NOESY spectrum, new peaks will show up connecting amide protons and C-alpha's, connecting C-alpha's and C-beta's, and connecting amides. The only NOESY data that we have shows the region with specific amide-C-alpha connectivities. Here we will see the i to i+3 cross peaks, characteristic of an alpha helix (ie., arising because those protons are brought into close proximity by the structure of the alpha helix. 4.3-8.22 / 4.76-8.55

C-alpha-amide peaks can also be observed for i to i+4, but they are weaker.



Work this problem **by Noon Tuesday, April 2** and you can check your *sequence* with Craig. If your sequence is off you may "buy" the sequence for 10 points. In any case, you should assign all protons in the spectrum.



In the plot below, peaks new to NOESY (circled) tell us about sequence connectivity.