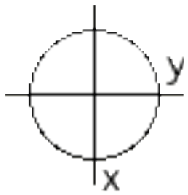
This is an assignment, due in class, 9:30am, Thursday, April 3

You will get credit for putting in a substantial effort on this
Answer key will be available on line on April 3

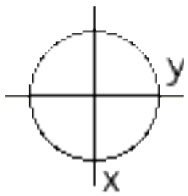
1. (5 points) Show, on the laboratory frame spin vector diagrams below, what will happen for each pulse sequence (ie. what is the state of the single magnetization vector precessing at ω_o immediately after the sequence?).

   Note: $\tau_D = \frac{\pi}{2\omega_1}$ The applied field lies along the *z*-axis, as usual.

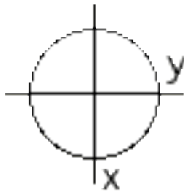
   a) $-90°_x - 90°_x -$

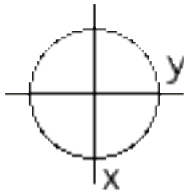
   b) $-90°_x - \tau_D - 90°_x -$

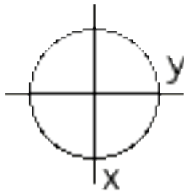
   c) $-90°_x - 2\tau_D - 90°_x -$

   d) $-90°_x - \tau_D - 180°_y -$

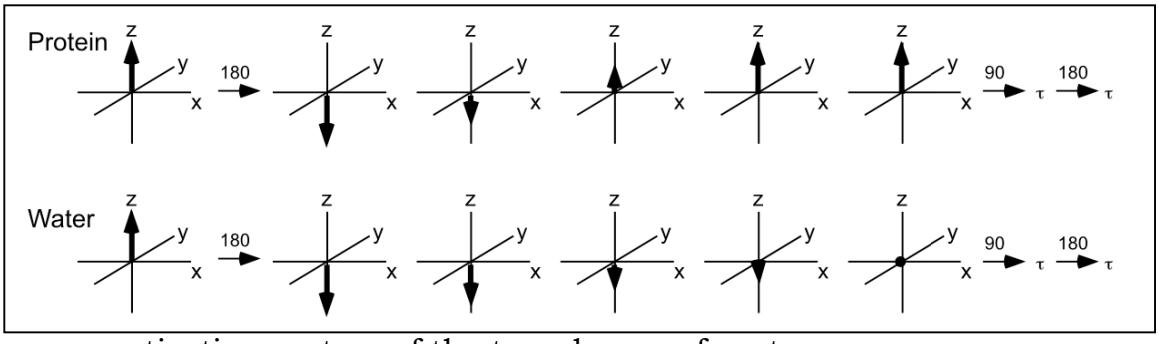
   e) $-90°_x - 2\tau_D - 180°_y -$

2. (5 points) In a ^1H 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?

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.

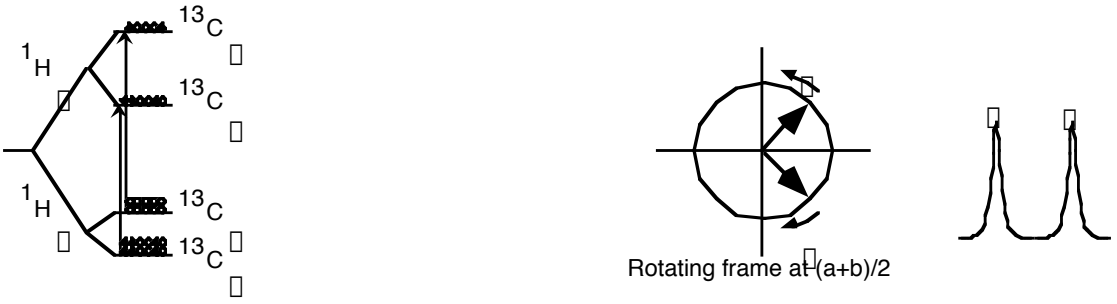
4. (20 points) The energy levels for a singlet ^{1}H split by a singlet ^{13}C are shown below. The difference in populations between the levels is illustrated qualitatively. For each of the following situations (a-d), draw:

   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.



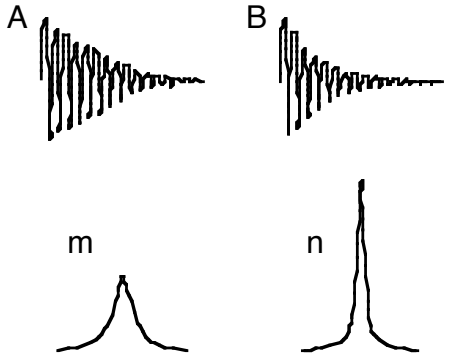
   a) Selective saturation (decoupling) of transition a (only).

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

c) 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.

5. Answer the following questions:

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



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.

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.

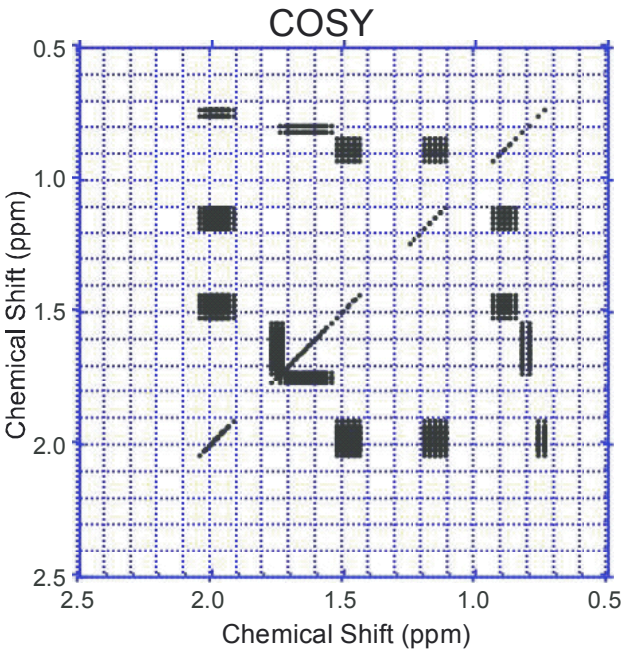
   a) (20 points) Assign the signals in each spectrum to individual amino acids

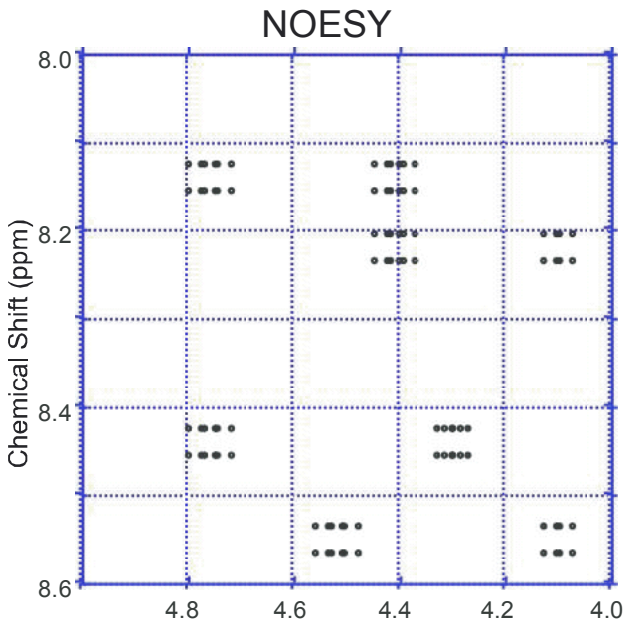
      On each spectrum, circle each cross peak group and write its assignment (eg. circle a multiplet and label it Asp-CαH in one direction and CβH in another).

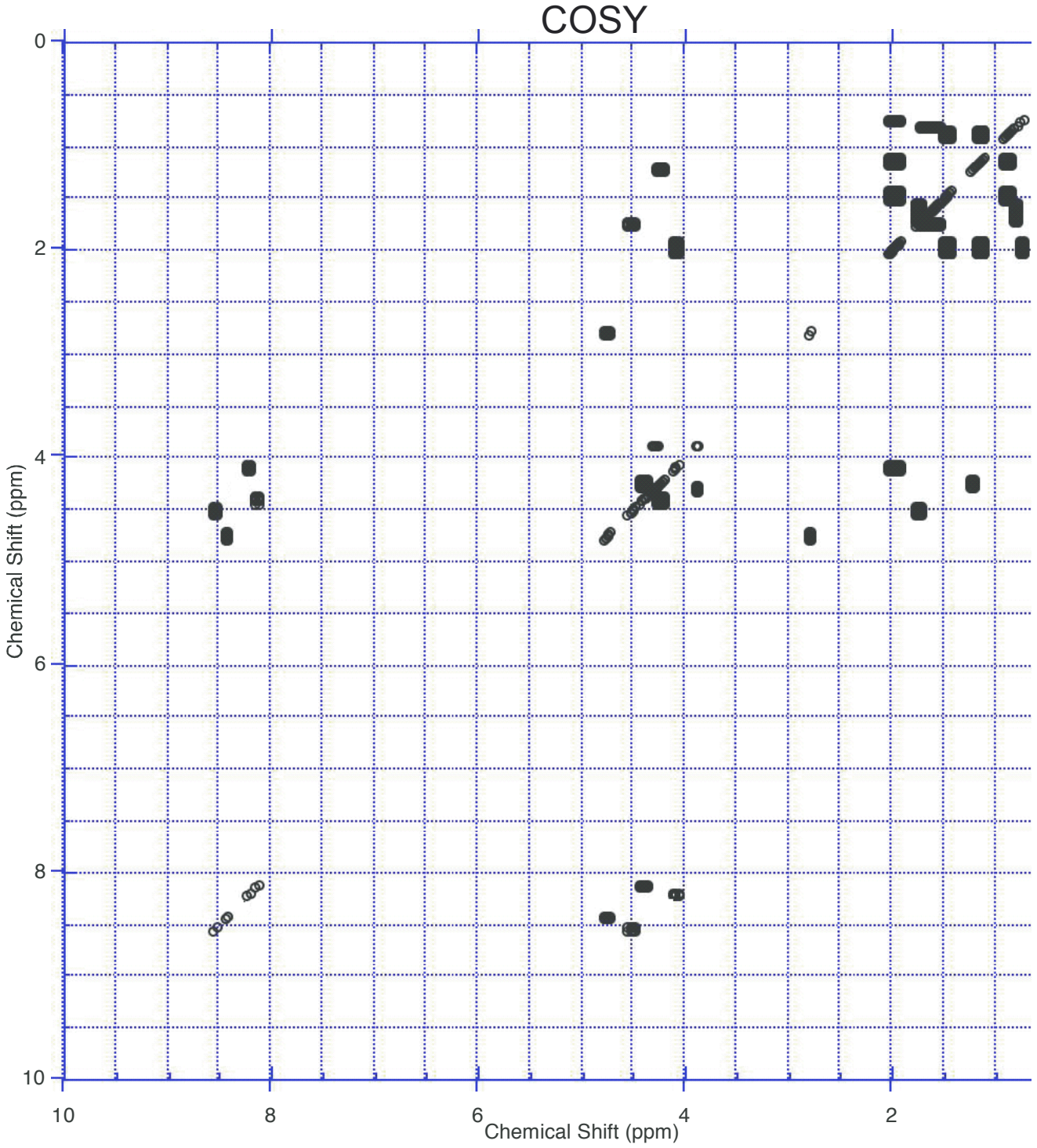
   b) (20 points) Determine the sequence of the peptide, to the extent that you can. Comment on any amibuities.

   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?

   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.


COSY
0.5
1.0
1.5
2.0
2.5
Chemical Shift (ppm)
2.5
2.0
1.5
1.0
0.5
Chemical Shift (ppm)


NOESY
8.0
8.2
8.4
8.6
Chemical Shift (ppm)
4.8
4.6
4.4
4.2
4.0


COSY
0
2
4
6
8
10
Chemical Shift (ppm)
10
8
6
4
2
Chemical Shift (ppm)