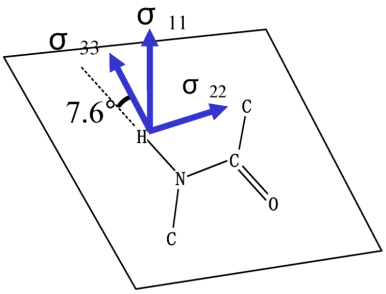
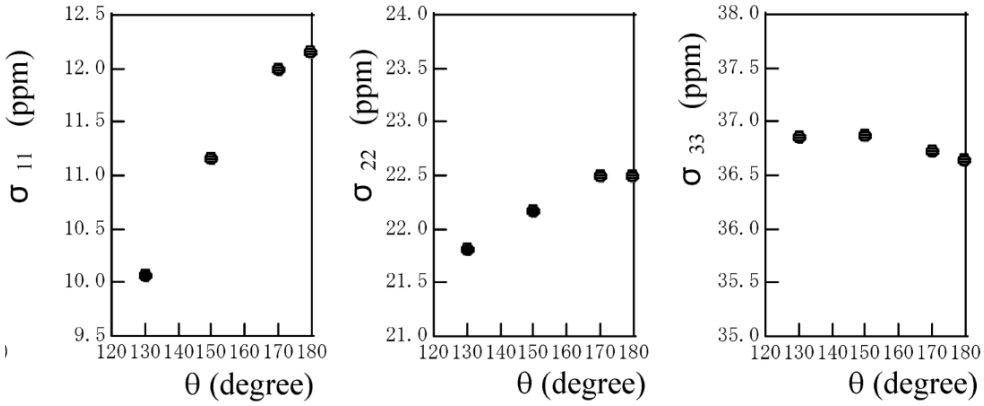
Name _____

Final Exam: CHEM/BCMB 8190 (228 points) Monday, 1 May, 2017

INSTRUCTIONS: You will have three hours to work on this exam. You can use any notes or books that you bring with you to assist you in answering the questions. You cannot leave the examination room to retrieve additional notes or books. No electronic devices of any kind are allowed, except for a hand-held calculator. No access of any kind to the internet is allowed. Please write your answers on this exam in the space provided. Make certain to write your name on the exam. Please do not detach any pages from the exam (leave it stapled and intact). *If a question asks for a calculation, simply 'setting up' the calculation or writing down an equation is not adequate. You must complete the calculation for credit.* There is a table at the end of the exam with information you may find useful.

1) The shielding tensor elements for the hydrogen-bonded amide proton of N-methylacetamide (hydrogen bonded to formamide) were calculated using *ab initio* molecular orbital methods as a function of the hydrogen bond angle (*θ*). The directions of the chemical shielding tensor components (σ_{11}, σ_{22} and σ_{33}), along with the chemical structure, are shown in the figure (right). The values of the tensor elements as a function of *θ* are shown in the plots below.





If, in solution, the hydrogen bond angle is 150°, and the chemical shift of this amide ^{1}H nucleus is 9.0, what would be the chemical shift of this ^{1}H nucleus in solution if the hydrogen bond angle was 170° instead of 150°? You will have to show your work for credit. (**8 points**)

The isotropic (solution) tensors (in ppm, as in the plots) are:

$$\sigma_{iso,150} = \mathrm{Tr}\begin{bmatrix} 11.2 & & \\ & 22.2 & \\ & & 36.8 \end{bmatrix} = (11.2 + 22.2 + 36.8)/3 = 23.4 \qquad \sigma_{iso,170} = \mathrm{Tr}\begin{bmatrix} 12.0 & & \\ & 22.5 & \\ & & 36.7 \end{bmatrix} = (12.0 + 22.5 + 36.7)/3 = 23.7$$

The chemical shift difference is then 23.4 - 23.7 = -0.3 ppm. So, at 170° the hydrogen is 0.3 ppm more shielded, so its chemical shift is 8.7 ppm.

Name _____

2) A protein contains the following unassigned amino acid sequence:

….. N P T E A E L Q D M I N E V D A …..

You have data from several triple resonance experiments for the residues in this unassigned region, and the data is complete (no missing peaks). For each amide $^{1}H_{i}$, $^{15}N_{i}$ pair, you have correlated the $^{13}C\alpha_{i}$, $^{13}C\beta_{i}$, $^{13}C\alpha_{i-1}$, $^{13}C\beta_{i-1}$, $^{1}H\alpha_{i-1}$, $^{1}H\beta_{i-1}$, and $^{13}C'_{i-1}$ (carbonyl) chemical shifts.

a) For residue *j*, the following correlations are observed:

$^{13}C\alpha_{j}$ / $^{13}C\beta_{j}$ = 64.1 ppm / 39.1 ppm

$^{13}C\alpha_{j-1}$ / $^{13}C\beta_{j-1}$ / $^{13}C'_{j-1}$ = 57.0 ppm / 35.8 ppm / 178.3 ppm

Which amino acid in the sequence is residue *j* ? You will have to provide a proper explanation for credit. (**6 points**)

Isoleucine. For the amino acid sequence shown, the only residues whose chemical shift ranges for $^{13}C\alpha$ and $^{13}C\beta$ are consistent with those shown for two adjacent amino acids are methionine (j-1) and isoleucine (j).

b) What do you think is the secondary structure of the region of the protein that residue *j* is in? Why (you will have to justify your answer for credit)? (**4 points**)

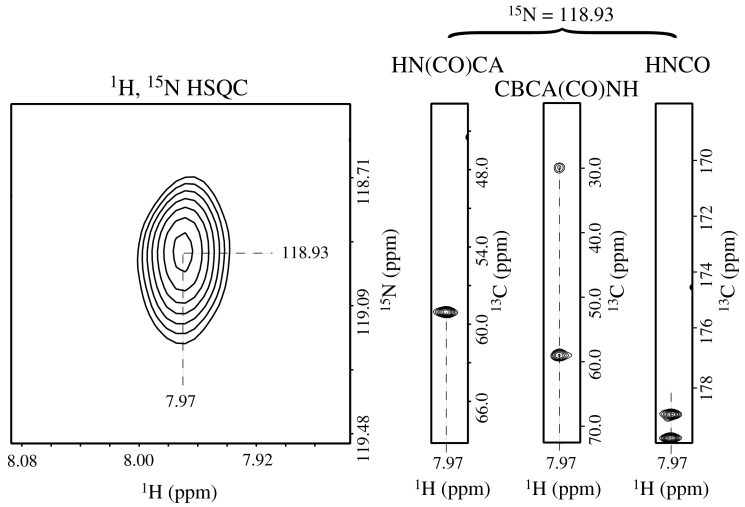
Alpha helix. The $^{13}C\alpha$ chemical shifts for the isoleucine and the methionine residue that preceeds it in the sequence are upfield shifted from the random coil values.

c) For amino acid *k*, you have correlated $^{1}H_{k}$, $^{15}N_{k}$, $^{13}C\alpha_{k-1}$, $^{13}C\beta_{k-1}$ chemical shifts. In order to link residue *k* to the preceding residue in the sequence, you need to find a ^{1}H, ^{15}N pair with intraresidue $^{13}C\alpha$ and $^{13}C\beta$ correlated chemical shifts that match $^{13}C\alpha_{k-1}$, $^{13}C\beta_{k-1}$. Even though your data is complete (no missing peaks), you cannot find these matches. What is the identity of residue *k*? You will have to provide a proper explanation for credit. (**6 points**)

Threonine. The previous residue has to be proline, which does not have an amide hydrogen, thus no possibility for an H-N pair to correlate with its own $^{13}C\alpha$ and $^{13}C\beta$.

Name _____

3) Regions from two- and three-dimensional heteronuclear and triple resonance experiments for a particular protein are shown. The displayed regions are plotted near the baseline, and the entire ^{13}C spectral widths are shown for the triple resonance data sets (i.e. no peaks are "missing" from the spectra). Please provide a good explanation for why two signals are observed in the displayed region of the HNCO spectrum. (**6 points**)



Two residues in the protein, i and j, have nearly identical main chain amide ^{1}H and ^{15}N chemical shifts, and the residues that precede them (i-1 and j-1) have nearly identical $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ chemical shifts. The ^{13}C chemical shifts for the carbonyl carbons of i-1 and j-1 are, however, significantly different from one another.

4) When assigning the side chain resonances for a particular Val residue in a protein using amide resolved TOCSY spectra (C(CO)NH and H(CCO)NH), peaks were found with the following chemical shifts:

C(CO)NH: ^{13}C = 21.0 ppm, 22.0 ppm H(CCO)NH: ^{1}H = 0.8 ppm, 0.9 ppm

a) What nuclei of the Val residue can these chemical shifts be assigned to? Please explain for credit? (**4 points**)

These are the chemical shifts of the methyl groups, which are distinct from those of other nuclei in Val residues.

b) What additional information could potentially be obtained for these shifts/nuclei from an HCCH-TOCSY experiment? (**4 points**)

The HCCH-TOCSY experiment would allow correlation of each ^{13}C shift with the ^{1}H shift of its attached protons.

5) In a recent publication, Arora et al. (*Nat. Struct. Biol*. **8**, 334-338 (2001)), determined the three-dimensional fold (low-resolution structure) of a membrane protein (19 kDa, 177 amino acids) in micelles. The protein (transmembrane domain of OmpA) that they used was nearly completely (~98%) deuterated at nonexchangeable sites.

a) Why, in general, are proteins sometimes deuterated for NMR studies? For credit you must explain any assertions you make. (**4 points**)

For large proteins or complexes, the rotational correlation time is large, and the T_2 relaxation times for ^{13}C nuclei are very short. During heteronuclear experiments, ^{13}C magnetization decays very quickly, resulting in substantial signal losses. Deuteration increases T_2 of ^{13}C by removing the efficient dipolar ^{1}H-^{13}C relaxation mechanism.

b) Given the relatively small size of OmpA, why did the researchers use deuterated OmpA? (**6 points**)

Although the protein is rather small, the complex of the OmpA domain and the micelle is very large, with a corresponding large rotational correlation time, leading to very short T_2 values for the ^{13}C nuclei in the protein.

c) In their assignment procedure, the researchers used an experiment called HN(CA)CB. What nuclei are correlated in this experiment, and how does this differ from the HNCACB experiment? (**4 points**)

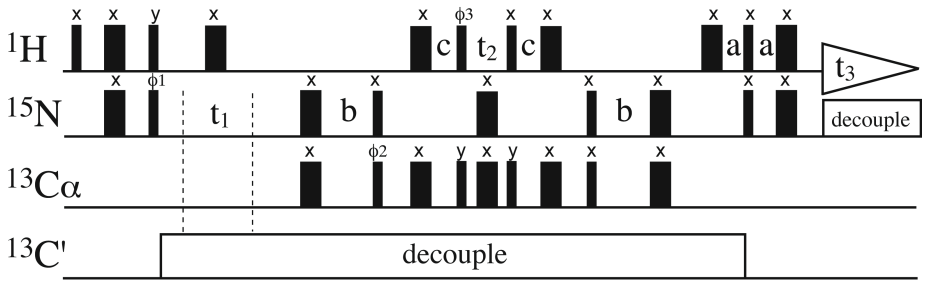
Given the name, the HN(CA)CB experiment would correlate amide ^{1}H and ^{15}N chemical shifts with $^{13}C\beta$ chemical shifts. $^{13}C\alpha$ chemical shifts are not correlated, as they are in the HNCACB experiment.

d). The authors did not use the CBCA(CO)NH experiment in their assignment strategy. Why? (**6 points**)

The CBCA(CO)NH experiment would not work because the protein is nearly 100% deuterated (this experiment begins with creation of transverse $^{1}H\alpha$ and $^{1}H\beta$ magnetization).

Name _____

6) Consider the three-dimensional, triple resonance pulse sequence for correlating chemical shifts in isotopically labeled proteins shown below:



a) For amino acid *i* in a protein, the chemical shifts for what nuclei in *i*, or adjacent amino acids, are correlated, or might be correlated, by this experiment? You will have to provide a proper explanation for credit. (**8 points**)

$^1H^N$ magnetization is transferred to ^{15}N via INEPT, with a ^{15}N evolution period (t_1), then transferred to $^{13}C^{\alpha}_i$, and most likely $^{13}C^{\alpha}_{i-1}$ also (no ^{13}C evolution), via INEPT, then transferred via INEPT to $^1H^{\alpha}_i$ and $^1H^{\alpha}_{i-1}$ with an evolution period for ^{1}H, then returned via the same path to $^1H^N$, with detection of $^1H^N$. So, ^1H_i, $^{15}N_i$, $^1H^{\alpha}_i$, $^1H^{\alpha}_{i-1}$ are correlated.

b) In the pulse sequence are two time periods labeled 'a' and 'c'. What is the ratio of 'a' to 'c'? Please explain for credit. (**6 points**)

These time periods are part of INEPT transfer periods. These must be set to 1/(4J). Period 'a' is 1/(4J_{HN}) for the amide H-N coupling, and period 'c' is 1/(4J_{CH}) for the alpha H-C coupling. So, the ratio a/c=$^1J_{HC}/^1J_{HN}$.

c) What would be a good name (acronym) for this experiment? Please explain why. (**4 points**)

HN(CA)HA. ^1H_i, $^{15}N_i$, $^1H^{\alpha}_i$, $^1H^{\alpha}_{i-1}$ are correlated, and the magnetization transfer proceeds via $^{13}C^{\alpha}$, hence (CA).

d) Would you expect this experiment to be more or less sensitive than the HNCO experiment? than the HNCA experiment? Please explain for credit. (**4 points**)

Less sensitive than both. HNCO is the most sensitive triple resonance experiment. HNCA has one less magnetization transfer step than this experiment, so would be more sensitive.

e) Triple resonance assignment experiments often come in pairs, for reasons we discussed in class. Examples are HNCA/HN(CO)CA, HNCACB/CBCA(CO)NH, for instance. Among those triple resonance experiments that we discussed in class, which one could serve to pair with the experiment above? (**4 points**)

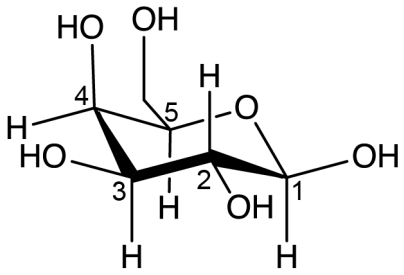
HBHA(CBCACO)NH, or perhaps H(CCO)NH (a TOCSY experiment)

Name _____

7) A hydrogen exchange experiment is conducted observing the intensity of amide proton resonances in a protein in H_2O as a function of time after dilution with D_2O. At pH 7.5 a proton has a half-life of 3 hr. Assuming an EX2 mechanism, what half-life do you expect at pH 6.5? You will have to explain for credit. (**8 points**)

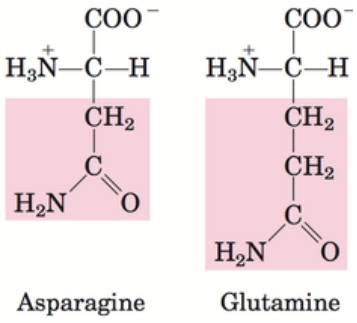
30 hours. Under EX2 conditions, exchange rate is pH dependent. The log of the exchange rate is linear with pH, so, a decrease in pH of 1 pH unit changes (decreases) the rate by a factor of 10, so, 30 hours.

8) Residual dipolar coupling contributions to splitting of carbon signals in the indirect dimension of a coupled ^{13}C-^{1}H HSQC spectrum of a galactose (below) containing oligosaccharide show values of +3.0 Hz, -5.0 Hz, -5.1 Hz and -5.3 Hz for cross peaks that will eventually be assigned to the H2, H3, H4 and H5 cross peaks (not necessarily in this order). Based on these data, what can you say about the relative orientations of the four C-H vectors, and which RDC is from H4? You will have to explain your reasoning for credit. (**6 points**)



The C-H bond vectors for positions 2, 3, and 5 are nearly parallel, so their dipolar couplings would be about the same (-5.0, -5.1, and -5.3 Hz, but not necessarily in that order). The coupling from the C-H bond vector for position 4, however, would be expected to be much different, as it is approximately perpendicular to the others. Hence, for position 4, the coupling is +3.0 Hz.

9) Which triple resonance experiment(s) could be used to correlate the ^{15}N chemical shifts of side chain $–CH_2$-(C=O)-NH_2 groups of glutamine or asparagine residues in proteins with side chain ^{13}C chemical shifts? Explain. (**6 points**)



The bonding of the sidechain atoms in these amino acids is essentially identical to that in a protein backbone. Any experiments that transfer magnetization through the carbonyl group would work. HN(CO)CA would correlate the $^{1}H^{N}$ and ^{15}N chemical shifts (of the sidechain -NH_2 group), with $^{13}C\beta$ of Asn and $^{13}C\gamma$ of Gln. CBCA(CO)NH would provide the same correlations as HN(CO)CA for Asn, and for GLN would, additionally, correlate the $^{13}C\beta$ chemical shift with $^{13}C\gamma$, $^{1}H^{N}$, and ^{15}N. The HBHA(CBCACO)NH experiment would provide the correlations to the side chain ^{1}H nuclei. The C(CO)NH (TOCSY) experiment would, for both amino acids, provide the same correlations as CBCA(CO)NH. The H(CCO)NH (TOCSY) experiment, likewise, could be used for correlations to the ^{1}H nuclei of the sidechains. The HNCA experiment could also be used to provide the same correlations as the HN(CO)CA experiment as long as the delays in the INEPT transfer from ^{15}N to ^{13}C were set appropriately (two-bond coupling of ^{15}N with $^{13}C\beta$ of Asn or $^{13}C\gamma$ of Gln). The HNCO experiment also could be used.

Name _____

10) The one bond ^{15}N-$^{13}C\alpha$ coupling constant in a peptide is 7 Hz. What do you expect the ^{15}N=^{13}C one bond coupling constant in a histidine sidechain ring to be? Provide a calculation and explanation for credit. (**8 points**)

The magnitudes of one-bond couplings are dependent on (proportional to) the fraction of s character in the bond. For ^{15}N-$^{13}C^{\alpha}$, the carbon is sp^3 hybridized (1/4 s), and the nitrogen sp^2 (1/3 s), so 1/4×1/3=1/12. For ^{15}N=^{13}C, both atoms are sp^2 hybridized, so 1/3x1/3=1/9. So, based on a value of 7 Hz for ^{15}N-$^{13}C\alpha$, the predicted value for ^{15}N=^{13}C would be 7x12/9=9.3 Hz.

11) Would you expect the ^1H signal of CHF_3 to be up field or down field of a CH_4 resonance? Please explain why for credit. (**4 points**)

Fluorine is highly electronegative. The inductive effect would tend to highly deshield the hydrogen in CHF_3 relative to the hydrogens in CH_4. Thus, the ^1H signal in CHF_3 would be downfield relative to the ^1H signal of CH_4.

12) Sketch the ^1H NMR signal for CHF_3. If it is a singlet, please explain why. If it is a multiplet, please explain the multiplet pattern, the relative intensities of the peaks, and what the significance is of the distance between the peaks. (**6 points**)

The signal is a quartet due to coupling to the three ^{19}F (spin ½) nuclei. There are four possible energy levels for the ^1H nucleus: all coupled ^{19}F nuclei in the α state ($\alpha\alpha\alpha$), all coupled ^{19}F nuclei in the β state ($\beta\beta\beta$), two of the coupled nuclei in the α state ($\alpha\alpha\beta$, $\alpha\beta\alpha$, $\beta\alpha\alpha$), and two of the coupled nuclei in the β state ($\beta\beta\alpha$, $\beta\alpha\beta$, $\alpha\beta\beta$). The outer components of the multiplet result from the first two of these, the inner components from the latter two. Because there are three possibilities for each of the inner components, the intensities of these are three times the intensities of the outer components (relative intensities of the quartet peaks 1:3:3:1). The distance between any adjacent pair of peaks represents the magnitude of the ^1H-^{19}F coupling constant (in Hz).

Name _____

13) Calculate the direct product (Kronecker product) of the two matrices below. (**4 points**)

$$A = \begin{bmatrix} 1 & -2 \\ -1 & 0 \end{bmatrix} \qquad B = \begin{bmatrix} 4 & -3 \\ 2 & 3 \end{bmatrix}$$

$$A \otimes B = \begin{bmatrix} 1\times 4 & 1\times -3 & -2\times 4 & -2\times -3 \\ 1\times 2 & 1\times 3 & -2\times 2 & -2\times 3 \\ -1\times 4 & -1\times -3 & 0\times 4 & 0\times -3 \\ -1\times 2 & -1\times 3 & 0\times 2 & 0\times 3 \end{bmatrix} = \begin{bmatrix} 4 & -3 & -8 & 6 \\ 2 & 3 & -4 & -6 \\ -4 & 3 & 0 & 0 \\ -2 & -3 & 0 & 0 \end{bmatrix}$$

14) Schemes based on relaxation interference mechanisms (cross-correlation effects) can dramatically improve resolution for large molecules at high magnetic field strengths. It has been reported that signals from aromatic rings (phenylalanine) can benefit significantly from these effects (otherwise, these signals are broad, and due to chemical shift degeneracy are often overlapped and difficult to interpret). For C-H groups in phenylaline rings, provide an explanation for these effects. You should include a discussion of what effects are involved, how they interact (interfere), and what the result is. (**8 points**)

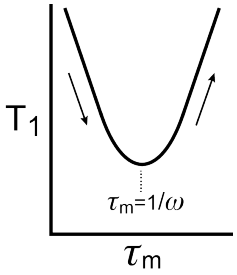
There is a substantial CSA contribution to relaxation of ^{13}C nuclei in aromatic rings. The most shielded region is perpendicular to the ring (above and below), and the least shielded in the plane of the ring, and along the ^{13}C-^{1}H bonds. In addition, for ^{13}C-^{1}H, there is the always-strong ^{13}C-^{1}H dipolar interaction that efficiently relaxes ^{13}C nuclei, and there is no exception for aromatic rings. Thus, the mechanism for relaxation interference in aromatic rings that results in improved resolution is most likely interference between CSA and dipole-dipole mechanisms.

Name _____

15) A molecule with a correlation time of 3 ns is found to have an ^{15}N spin lattice relaxation time of 0.5 s at 18.7 T (800 MHz for ^{1}H) for a protonated amide site. Will the relaxation time increase or decrease as we lower the temperature? You will have to do a calculation (show your work) and justify your reasoning for credit. (**8 points**)

$$\frac{1}{T_1} \propto D \times 3J(\omega_N) = D3\frac{2}{5}\left(\frac{S^2\tau_m}{1+\omega_N^2\tau_m^2}\right) = D\frac{6}{5}S^2\left(\frac{\tau_m}{1+\omega_N^2\tau_m^2}\right) = C\left(\frac{\tau_m}{1+\omega_N^2\tau_m^2}\right) \quad T_1 = \frac{1}{C}\frac{1+\omega_N^2\tau_m^2}{\tau_m} = \frac{1}{C}\frac{1}{\tau_m} + \frac{1}{C}\omega_N^2\tau_m$$

$$\frac{d(T_1)}{d\tau_m} = -\frac{1}{C}\frac{1}{\tau_m^2} + \frac{1}{C}\omega_N^2 \qquad \frac{1}{C}\frac{1}{\tau_m^2} = \frac{1}{C}\omega_N^2 \qquad \frac{1}{\tau_m^2} = \omega_N^2 \qquad \frac{1}{\tau_m} = \omega_N \qquad \tau_m = \frac{1}{\omega_N}$$



The equation above describes the curve of T_1 versus τ_m. The function has a minimum at $\tau_m=1/\omega$ (see derivative above). At low τ_m, T_1 decreases as τ_m increases (for small increases in τ_m, i.e. as long as τ_m does not increase above $1/\omega$). At high τ_m, T_1 increases as τ_m increases. Here, the value of γ for ^{15}N is approximately 1/10 that of ^{1}H, so $\omega_N=2\times\pi\times80\times10^6=5.03\times10^8$ and $1/\omega_N=1.99\times10^{-9}$. The value of τ_m is 3×10^{-9}, which is greater than $1/\omega$, so, if τ_m increases, T_1 increases. Lowering the temperature increases τ_m (Stokes law, increased viscosity increases the correlation time), so T_1 increases.

16) The following is the density matrix for a pair of ^{1}H nuclei at a time just prior to acquisition. What is the value of the *y*-magnetization at the beginning of acquisition. You will have to do the appropriate calculation and show your work for credit (you can leave out the constants). (**8 points**)

$$\sigma(t) = \begin{bmatrix} 0.5 & 0.1 & 0.1i & 0.5 \\ 0.1 & 0 & 0.2 & 0.1i \\ -0.1i & 0.2 & 0 & 0.1 \\ 0.5 & -0.1i & 0.1 & -0.5 \end{bmatrix}$$

Because we know the density matrix (at a particular time, just before acquisition), we can calculate the y-magnetization as:

$$M_y(t) = \mathrm{Tr}\left\{\left[\hat{\boldsymbol{\sigma}}(t)\right]\left[\hat{\boldsymbol{\mu}}_y\right]\right\}$$

Because we have two spins, the magnetic moment operator (leaving out constants) will be the sum of the operators for $\hat{\boldsymbol{I}}_{1y}$ and $\hat{\boldsymbol{I}}_{2y}$:

$$\hat{\boldsymbol{I}}_{1y} + \hat{\boldsymbol{I}}_{2y} = \begin{bmatrix} 0 & 0 & -i & 0 \\ 0 & 0 & 0 & -i \\ i & 0 & 0 & 0 \\ 0 & i & 0 & 0 \end{bmatrix} + \begin{bmatrix} 0 & -i & 0 & 0 \\ i & 0 & 0 & 0 \\ 0 & 0 & 0 & -i \\ 0 & 0 & i & 0 \end{bmatrix} = \begin{bmatrix} 0 & -i & -i & 0 \\ i & 0 & 0 & -i \\ i & 0 & 0 & -i \\ 0 & i & i & 0 \end{bmatrix}$$

$$M_y(t) \propto \mathrm{Tr}\left\{\left[\hat{\boldsymbol{\sigma}}(t)\right]\left[\hat{\boldsymbol{\mu}}_y\right]\right\} = \mathrm{Tr}\left\{\begin{bmatrix} 0.5 & 0.1 & 0.1i & 0.5 \\ 0.1 & 0 & 0.2 & 0.1i \\ -0.1i & 0.2 & 0 & 0.1 \\ 0.5 & -0.1i & 0.1 & -0.5 \end{bmatrix}\begin{bmatrix} 0 & -i & -i & 0 \\ i & 0 & 0 & -i \\ i & 0 & 0 & -i \\ 0 & i & i & 0 \end{bmatrix}\right\} = \mathrm{Tr}\begin{bmatrix} 0.1i+0.1i^2 & & & \\ & -0.1i+0.1i^2 & & \\ & & 0.1i+0.1i^2 & \\ & & & 0.1i^2-0.1i \end{bmatrix}$$

$$M_y(t) \propto 4(0.1i^2) = -0.4$$

Name _____

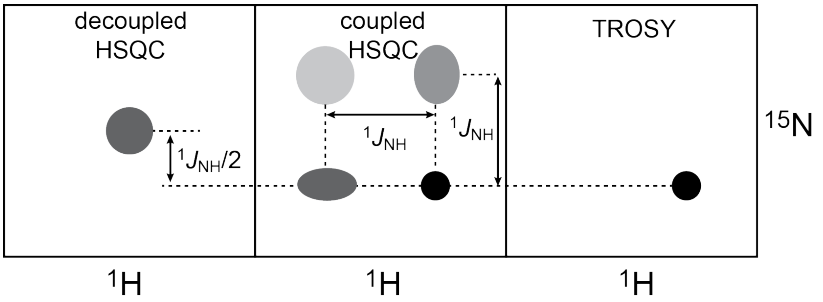
17) The T_2 of ^{15}N in an HSQC experiment conducted using a 500 MHz magnet (11.75 T) is 0.1s. Signals in the ^{15}N dimension are spread over 30 ppm. How many complex t_1 points should you collect? (**6 points**)

The γ for ^{15}N is ~10 fold lower than for ^{1}H, so, at 11.75 T, the ^{15}N frequency is ~50 MHz, so 30 ppm is 1500 Hz, which is the minimal sweep width (SW) that allows observation of all ^{15}N signals that are present. The dwell time (DW) is $1/(2\times SW)=1/(2\times 1500)=3.33\times 10^{-4}$ s. A good rule of thumb is to collect data for an acquisition time (AT) of $2\times T_2$, so if T_2 is 0.1s, then here the acquisition time is $2\times 0.1=0.2$ s. The acquisition time is equal to the number of points collected (NP) multiplied by DW, $NP=AT/DW=0.1/(3.33\times 10^{-4})=300$. So, 300 points should be collected.

18) In an SAR by NMR two ligands are identified that bind to adjacent sites on a protein with binding constants of 1500 M^{-1} and 2000 M^{-1} respectively. They are then successfully linked for form a single ligand. What would you expect for an approximate binding constant? (**4 points**)

The binding constant for the linked inhibitors is the product of the binding constants for the individual (not linked) inhibitors. Here, $1500\times 2000=3\times 10^{6}$ M^{-1}.

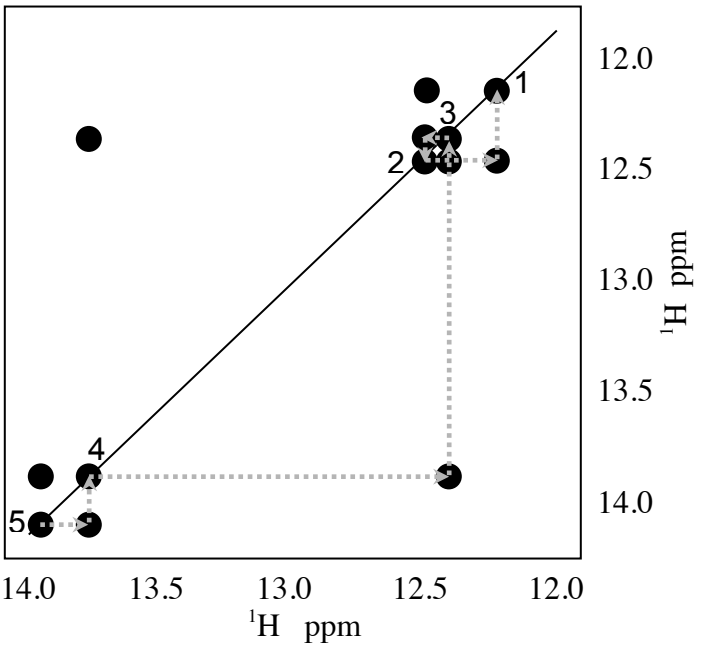
19) A TROSY version of an ^{15}N-^{1}H HSQC spectrum is collected on a large protein. How do the positions of peaks in this spectrum relate to positions in a normal HSQC? For credit you should draw a sketch and indicate distances between signals/peaks that are relevant. (**6 points**)



Name _____

20) The spectrum (right) represents the imino portion of a ^{1}H-^{1}H NOESY on the following RNA molecule that dimerizes to form an A form helix. From left to right, the first 5 bases are labeled/numbered (as shown below):

1 2 3 4 5
GGCUUAAGCC



a) Give a probable assignment for the five signals shown in the NOESY spectrum. You should label the diagonal peaks with the correct assignment (1, 2, 3, 4, or 5). For credit, you must also explain your reasoning. (**10 points**)

The ^{1}H chemical shifts for AU pairs are further downfield. Therefore, 4 and 5 would be expected to be the downfield signals. Because 5 can have only one crosspeak (to 4), it would be the signal furthest downfield. Likewise, the signal for 1 can have only 1 crosspeak (to 2), therefore, the signal from 1 is that which is the furthest upfield signal. Tracing through the crosspeaks gives the remaining assignments.

b) How do chemical shift positions help in assignment for RNA molecules such as this? (**4 points**)

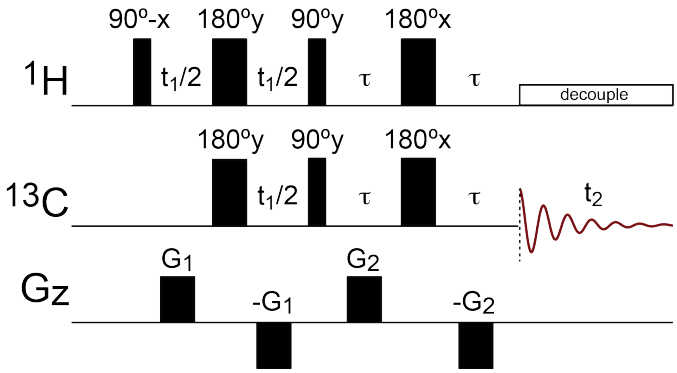
The ^{1}H chemical shifts for AU pairs are further downfield.

c) How would information from a ^{15}N-^{1}H HSQC help in assignment for RNA molecules such as this? (**4 points**)

For U bases, the ^{15}N chemical shifts are further downfield.

Name _____

21) The pulse sequence shown (right) is of a 2D experiment used for molecules with ^{1}H bound directly to ^{13}C (^{1}H-^{13}C). The delay τ is set to $1/(4J_{CH})$.



a) Just after the final 90° pulse on ^{13}C, from a product operator analysis, what are the important terms (ignore the pulsed field gradient pulses). (**8 points**)

$$-2\mathbf{I}_z\mathbf{S}_y \sin(2\pi J_{IS} t_1)$$

b) Based on your answer to 'a', what is measured in t_1 of this experiment? (**6 points**)

J_{IS}, i.e. $^{1}J_{CH}$

c) The pulsed field gradients are used to select for a pathway from ^{1}H to ^{13}C magnetization, while suppressing signals from ^{1}H nuclei attached to ^{12}C. The magnitudes of the first two gradient pulses are G_1 and the magnitudes of the second two are G_2. What should be the ratio of G_2 to G_1 for this pulse sequence to work as designed? (**6 points**)

For maximum signal, $\gamma_H G_1$ must be equal to $\gamma_C G_2$. Because $\gamma_H/\gamma_C \approx 4$, G_2 must be about 4 times as large as G_1

d) What is the purpose of the final τ-(^{1}H 180°x/^{13}C 180°x)-τ element of the pulse sequence? Please explain what is accomplished during this element and why it is included. (**6 points**)

Just after the final 90° pulse on ^{13}C, the magnetization of interest is antiphase ^{13}C magnetization, which would give an antiphase doublet if data collection were initiated at that point, and broadband 1H decoupling could not be employed (signal would disappear). The final τ-(^{1}H 180°x/^{13}C 180°x)-τ element refocuses this magnetization, which allows broadband decoupling to be employed.

Name _____

22) The chemical shift range for ^{19}F (γ = 25.1815 x 10^7 rad Tesla^{-1} s^{-1}) is quite large; ~1300 ppm. For a static magnetic field strength of 9.4 Tesla, what is the frequency difference between two ^{19}F signals separated by 1300 ppm? You must do a calculation and show your work for credit. (**6 points**)

$$\upsilon_L = \left|\frac{\gamma}{2\pi}\right| B_0 = \frac{25.1815\times10^7}{2\pi}\times 9.4 = 377 \text{ MHz}$$

$$\Delta\delta = \frac{\Delta\upsilon}{377 \text{ MHz}}\times 10^6, \text{ so } \Delta\upsilon = \Delta\delta \times \frac{377\times10^6}{10^6} = 1300\times 377 = 490{,}100 \text{ Hz}$$

23) Proton line widths for non-exchanging amide protons in myoglobin are approximately 15 Hz. Hemoglobin is a tetramer of subunits that are approximately the same size as myoglobin. What would you expect for line widths of non-exchangeable amide protons in hemoglobin? Provide a complete justification of your answer for credit. (**6 points**)

$$\Delta\upsilon = \frac{1}{\pi T_2^*} \quad \frac{1}{T_2} \cong \frac{D}{2}\left(4J(0)\right) \cong \frac{D}{2}\left(4\frac{2}{5}\left(\frac{S^2\tau_m}{1+\omega^2\tau_m^2}\right)\right) \propto \tau_m \text{ at zero frequency}$$

$$\tau_m = \frac{4\pi\eta a^3}{3k_bT} \propto a^3 \propto MW$$

$$\Delta\upsilon \propto \frac{1}{T_2} \propto J(0) \propto \tau_m \propto a^3 \propto MW$$

NMR linewidths are proportional to $1/T_2^*$, and given that they tend to be large (magnetic field inhomogeneity component negligible), are proportional to $1/T_2$. For large proteins, $1/T_2$ is proportional to the spectral density at zero frequency (J(0)), which is proportional to τ_m. The Stokes equation relates τ_m to molecular size (volume, a^3), which is proportional to mass. So, the linewidths are MW. So, because hemoglobin is four times larger than myoglobin, the linewidths would be ~60 Hz.

24) Five seconds after application of a 90° pulse, only 10% of the original (equilibrium) magnetization has returned to *z*. What is the approximate T_1 relaxation time constant? You must do a calculation and show your work for credit. (**6 points**)

$$M_z = M_0\left(1-e^{(-t/T_1)}\right) \quad M_z/M_0 = 1-e^{(-t/T_1)} \quad 1-M_z/M_0 = e^{(-t/T_1)} \quad \ln\left(1-M_z/M_0\right) = -t/T_1$$

$$\ln\left(1-M_z/M_0\right) = -t/T_1 \quad T_1 = -t/\ln\left(1-M_z/M_0\right) = -5/\ln\left(1-0.1\right) = 47 \text{ s}$$

Name _____

THIS PAGE IS INTENTIONALLY BLANK: you can use it as ‘scratch’ if you like

You may find some of the information in this table useful:

Nuclide	Spin *I*	Natural abundance (%)	Relative sensitivity	Gyromagnetic ratio, γ (10^7 rad T^{-1} s^{-1})	NMR frequency (MHz @ B_0 = 2.3488 T)
^{1}H	1/2	99.985	1.00	26.7519	100
^{2}H	1	0.015	9.65×10^{3}	4.1066	15.351
^{3}H	1/2	~0	1.21	28.5350	106.664
^{10}B	3	19.58	1.99×10^{-2}	2.8747	10.746
^{11}B	3/2	80.42	0.17	8.5847	32.084
^{12}C	0	98.9	-	-	-
^{13}C	1/2	1.108	1.59×10^{-2}	6.7283	25.144
^{14}N	1	99.63	1.01×10^{-3}	1.9338	7.224
^{15}N	1/2	0.37	1.04×10^{-3}	-2.7126	10.133
^{19}F	1/2	100	0.83	25.1815	94.077
^{31}P	1/2	100	6.63×10^{-2}	10.8394	40.481
^{103}Rh	1/2	100	3.11×10^{-5}	-0.846	3.1474
^{195}Pt	1/2	33.8	9.94×10^{-3}	5.8383	21.499