

Name: _____

**BCMB/CHEM 8190, BIOMOLECULAR NMR
FINAL EXAM – 12/12/02**

Instructions: This is an open book, limited time, exam. You may use notes you have from class and any text book you find useful, but you must bring materials you plan to use with you to the exam; you cannot leave the room to get other materials. You may also use a calculator. You will have two and a half hours to complete the exam. Write your answers and name on the exam; turn it in at the end of the period.

Part 1 (100 pts). Give short answers to the following:

- 1) The four lines of a proton AB spectrum are at the following frequencies: 1032.5, 1038.0, 1040.0, 1045.5 (in Hz). What is the size of the scalar coupling between A and B?

- 2) If this were an AX spectrum, the chemical shifts would be 1035.25 and 1042.5 (in Hz); give a set of possible values for the AB case.

- 3) The nitrogen T2 relaxation times for C α sites in a protein to be studied using an HSQC experiment is 0.05s. Assuming we need an indirect dimension spectral width of 50 ppm at 11.7T (125 MHz for ^{13}C), approximately how many t1 points would you collect?

- 4) Residual dipolar couplings can be measured for both ^1H - ^{15}N interactions across an amide bond and ^1H - ^{13}C interactions across an α -proton- α -carbon bond in a large protein. The largest residual dipolar coupling for amide bonds is observed to be 50 Hz. Assuming N-H and C-H bond lengths are the same, what is the largest coupling you expect to see for H α -C α bonds?

- 5) Using SAR by NMR techniques, two ligands with binding constants of 3.0×10^3 and 2.0×10^4 , are found to bind in spatially proximate sites on the surface of a protein. If we successfully link them in a rigid framework that doesn't perturb their individual

binding and neglect entropy effects, what might you expect for a binding constant of the new ligand?

6) One bond scalar coupling between ^{13}C and ^1H in methane (H_4C) is approximately 125 Hz. What do you expect one bond scalar coupling between ^{13}C and ^1H in formaldehyde ($\text{H}_2\text{C}=\text{O}$) to be?

7) Which do you expect to be more shielded, a proton 3\AA above the plane of a phenylalanine ring or a proton in the plane but 3\AA outside the ring?

8) 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?

9) A powder pattern arising from dipolar coupling in a solid has two prominent intensity maxima. To what angle between the interaction vector and the magnetic field do these correspond?

10) Based on the shell model of the nucleus what fundamental particle would you expect to determine the magnetic properties of ^{11}B (atomic number 5) ?

11) In discussing the effect of pulsed field gradients we suggested that I_+ and I_- product operators are more convenient members of a product operator basis set than I_x and I_y . Give the matrix representation of I_- in a single spin $1/2$ system.

12) Chemical shift anisotropy contributes 30% of T2 spin relaxation to protonated carbons on a phenylalanine ring at 100 MHz. At what frequency will TROSY type narrowing of resonances maximize?

13) A NOESY-HSQC of an ^{15}N labeled protein shows strong HN-HN connections between sequential residues and $\text{H}\alpha$ -HN connections between $i \rightarrow i + 1$ and $i \rightarrow i + 3$ residues. What type of secondary structure does this part of the protein have?

14) Chemical shifts of two slowly exchanging imino protons in an RNA sample lie at 15.0 and 13.0 ppm respectively. To what types of base pairs are they likely to belong?

15) If you wanted to connect ribose H1' protons to base protons in a non-isotopically labeled RNA sample, what experiments would you use and what proton would you connect to in an adenine ring?

16) In using a simulated annealing approach to protein structure determination, strong medium and weak NOE peaks are converted to upper limits for distance constraints. Approximately what distances are used?

17) In a simulated annealing approach to protein structures name two types of experimental information that can be included in addition to NOE derived distance constraints?

18) In studying cation ion binding sites in nucleic acids, what ion can give directly observable signals using a basic HSQC experiment?

19) What paramagnetic ion can substitute for Mg^{2+} in nucleic acid studies and what effect does it have on resonances from nearby protons?

20) Rapid protonation and deprotonation of a histidine ring in a protein has left the single well resolved H2 resonance of the ring unusually broad. The additional line broadening at 500 MHz is estimated to be 5 Hz. What additional contribution would you expect at 800 MHz?

Part 2 (30 points) Answer the following showing your work.

You are doing the sequential assignment of a protein having the following sequence, VATNIAGSELFAGHQGD, using triple resonance experiments (HNCACB and CBCA(CO)NNH). You have found a residue in your protein with the following amide chemical shifts:

^1H amide: 8.08 ppm
 ^{15}N amide: 110.2 ppm

You have also found the following correlations to this amide in the ^{13}C dimension

From the CBCA(CO)NNH: 16.0 ppm and 55.2 ppm
From the HNCACB: 16.0 ppm, 44.4 ppm, and 55.2 ppm

Another amide group has the following shifts and correlations:

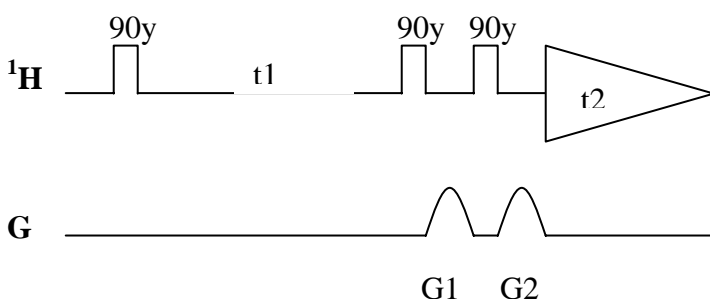
^1H amide: 8.21 ppm
 ^{15}N amide: 120.5 ppm

From the CBCA(CO)NNH: 38.3 ppm and 60.0 ppm
From the HNCACB: 16.0 ppm, 38.3 ppm, 60.0 ppm, and 55.2 ppm

- 1) What is the intra-residue $\text{C}\alpha$ shift for the first amide above? What is the most probable residue type?
- 2) Do the remaining correlations for the first amide belong to the $i-1$ or $i+1$ residue? What is the most probable residue type?
- 3) Given the correlations for the second amide what possible places could the three residue segment identified by these experiments fit into the sequence?

Part 3 (40 points) Answer the following showing your work.

As a part of the midterm we considered the behavior of a pair of coupled proton spins and the behavior of an isolated single proton spin under the influence of a two quantum filtered COSY experiment similar to that sketched below (one of the coupled spins and the isolated spin were on resonance with our observation frequency and didn't evolve due to chemical shift in t_1). Normally the experiment is used to remove signals from isolated spins, but in our previous example signals from both sets of spins were shown to remain. Now we have changed the sequence from using an x,x,y combination of pulse phases to a y,y,y combination, and have added pulsed field gradients (G1 and G2).

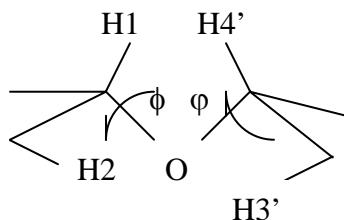


- a) First consider the sequence in the absence of gradients. Using product operators start with I_{z1} and I_{z3} before the first ^1H pulse (neglect magnetization associated with the second coupled spin at the start of the sequence). As before assume that the t_1 increment is set to a point where it equals $1/(2J)$ (J in Hz as opposed to radians). Show that for the new combination of pulse phases, magnetization from isolated spins and coupled spins have opposite signs (previously the magnetization had like signs).

- b) What would you do with the signals from the two sequences (x,x,y and y,y,y) to eliminate signals from the isolated spin?
- c) What do the product operators present after the second rf pulse and third rf pulse represent in terms of coherence orders?
- d) Now consider the addition of pulsed field gradient G1 and G2. If you wanted to observe in t_2 only parts of the density matrix that had existed as two quantum evolution during the period where gradient G1 was applied, what relative amplitude for gradient G2 would you choose?

Part 4 (30 points) Answer the following showing your work.

NOE data for a simple disaccharide are measured in both the presence and absence of a 100 kDa protein known to bind the disaccharide. The disaccharide has a glycosidic linkage between the O4 of one glucose ring and the anomeric carbon another glucose ring (see sketch). The ϕ angle (dihedral H1-C1-O- C4') is known to be relatively fixed at -60° . In the free state using a mixing time of 100 ms, weak positive NOEs are observed between the anomeric proton and the trans glycosidic H4', the anomeric proton and the trans glycosidic H3', and the anomeric proton and the H2 proton of its own ring in a ratio of 4:1:4. A scalar coupling of 2 Hz is also measured between a ^{13}C at the anomeric position (C1) and the ^1H proton at the 4' position.



- a) Given an intra ring H1-H2 distance of 2.7 \AA what are the trans glycosidic H1-H4' and H1-H3' distances assuming a rigid conformer?

- b) Given the following Karplus relationship for a three bond C-H coupling, what φ angles (C1-O-C4'-H4') are possible if a single rigid conformer exists? $J = 5.7\cos^2\varphi - 0.6\cos\varphi + 0.5$. Using the NOE data, which angle is most likely?
- c) In the presence of protein, using a mixing time of 100 ms, with the disaccharide in 10 fold excess, strong negative NOEs (about 50 times larger in magnitude) are observed in a ratio of 1:1:4. Suggest an approximate φ that might explain this change in NOEs (a sketch showing the change in φ would be adequate).
- d) Assuming a change in φ of 30° and assuming that the binding constant is sufficiently high to have the protein binding site saturated with disaccharide, what $^{13}\text{C}1\text{-}^1\text{H}4$ scalar coupling would you expect to observe in the presence of protein?
- e) Would the measurement of the coupling be useful in the situation described in (d) and why?