BMB 173 – Winter 2017

Homework Set 4.2 (150 points) – Assigned 2/2/17. Due 2/7/17

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Office Hours:

Friday 2/3/17 from 3-4pm in Spalding B123 or by appointment

Monday 2/6/17 from 11a-12pm in Spalding B123 or by appointment

1. **NMR Basics** (40 points)
   a. (5 points) What makes a nucleus NMR-active?
      i. An NMR-active nuclide will have a non-integer spin, and therefore a non-zero magnetic moment. So it has a magnetic dipole moment that can interact with the applied magnetic field.
      ii. A nucleus with spin=0 is not NMR active.
      iii. A nucleus with spin=1/2 is NMR active.
      iv. A nucleus with spin >1/2 is a quadrupolar nucleus and behaves differently than a spin=1/2 nucleus. For more information see https://en.wikipedia.org/wiki/Nuclear_quadrupole_resonance

Regularity of Nuclear Spin (I) for Different Nuclei:

Atomic Number	Mass Number	Nuclear Spin (I)
odd or even	odd	1/2, 3/2, ... 9/2
even	even	0
odd	even	1, 2, ... 6

   b. (5 points) What happens to a NMR-active atom in an applied magnetic field?
      i. A moving charged particle creates a magnetic field. This is the magnetic moment of the nucleus.
      ii. When this atom is placed in an external magnetic field, its magnetic dipole will align to the external field and precess at the Larmor frequency.

   c. (5 points) Why is a radiofrequency pulse used in an NMR experiment?
      i. After allowing the sample to align to the external magnetic field, a radiofrequency pulse is applied to disrupt the alignment. This induces "spin flips", forcing the sample into a higher energy level. Gradually the sample will relax back to alignment with the constant, external magnetic field. As it relaxes, it gives off the FID in the time domain.
      ii. The external magnetic field is along the z axis and the RF pulse pushes the net magnetic moment of the precessing nuclei into the XY plane where the signal can be read out by the detector. Gradually the relaxation brings the magnetic moments back into the Z direction.

   d. (5 points) What is the free induction decay (FID)?
      i. After taking a Fourier transform, the FID is in the frequency domain and yields the spectrum. A series of FID are collected and used to create the spectrum.
      ii. The FID is the current generated in the receiver coil by the magnetic moment.

e. (5 points) Why does the FID oscillate up and down?
   i. Alternating current.

f. (5 points) Why does the FID decay?
   i. The sample starts to relax back to equilibrium after the radiofrequency pulse.

g. (5 points) How is a Fourier transform used in NMR?
   i. Do a Fourier transform on the FID to figure out what frequencies are there.

h. (5 points) What do different frequencies in this spectrum correspond to?
   i. Different nuclei have different precession frequencies. The FID is sum of the sine waves representing the different frequencies in the signal.

2. **Understanding Chemical Shift** (35 points)

Chemical shift is defined as follows:

$$\text{Chemical shift, } \delta = \frac{\text{frequency of signal} - \text{frequency of reference}}{\text{spectrometer frequency}} [=] \frac{\text{Hz}}{\text{MHz}} [=] \text{ ppm}$$

a. (5 points) What would be the chemical shift of a peak that occurs 655.2 Hz downfield of the reference on a spectrum recorded using a 90 MHz spectrometer (in ppm)?

$\delta = \frac{655.2 \text{ Hz}}{90 \text{ MHz}} = 7.28 \text{ ppm}$ (this is the chemical shift of chloroform in $CDCl_3$)

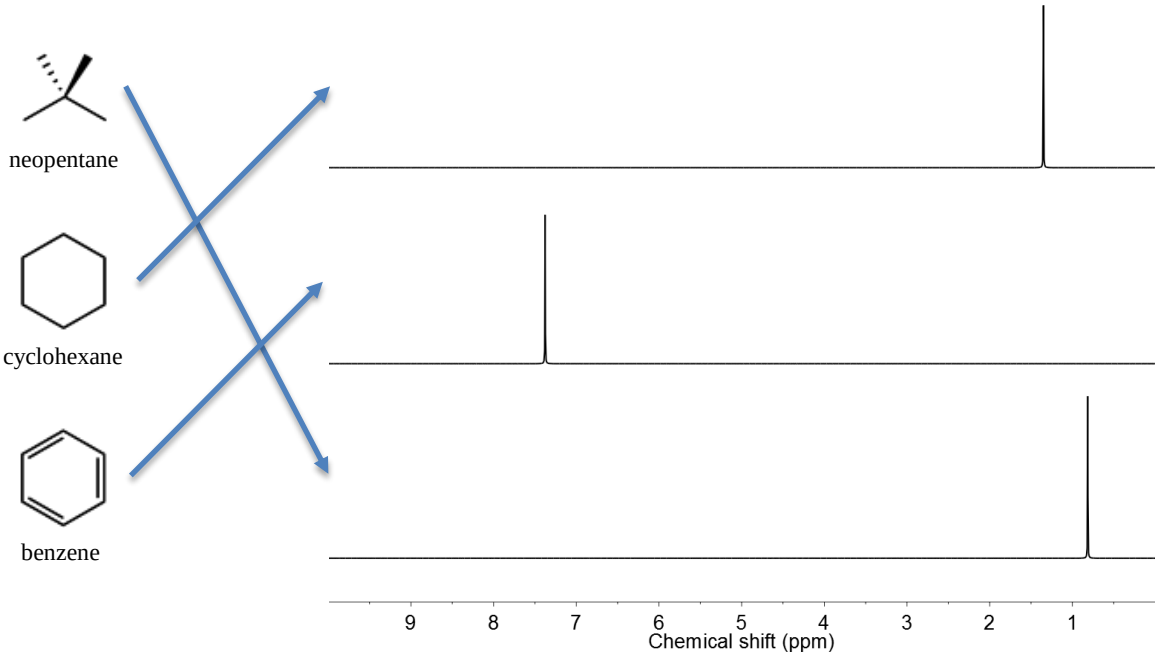
b. (5 points) What is the advantage of reporting the relative positions of NMR signals in ppm rather than absolute relative frequencies?

The frequency of an NMR signal is directly proportional to the strength of the magnet use to acquire the spectrum. Conversion to ppm simplifies the comparison of spectra acquired on different spectrometers. The shift in ppm will be the same!

c. (5 points) Given this, what frequency would you observe for the peak in part 'a' relative to the reference if the spectrum was recorded on a 300 MHz spectrometer?

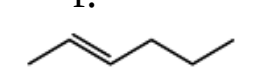
$\frac{x \text{ Hz}}{300 \text{ MHz}} = 7.28 \text{ ppm} \quad \rightarrow \quad x = 2184 \text{ Hz}$

d. (9 points) The approximate chemical shifts of protons can be predicted for different chemical groups found in proteins. The same holds for protons found in small molecules. Each of the following compounds exhibits a single ^{1}H NMR peak because the protons are chemically equivalent, and therefore will have the same resonance frequency in an NMR experiment. Using the predicted shifts from lecture, match each compound with its ^{1}H NMR spectrum.



e. (6 points) If protons are not chemically equivalent, they will have different resonance frequencies and multiple peaks will be present in the ^{1}H NMR spectrum. For each spectrum, circle the matching compound. Briefly explain your reasoning.

f.



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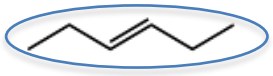
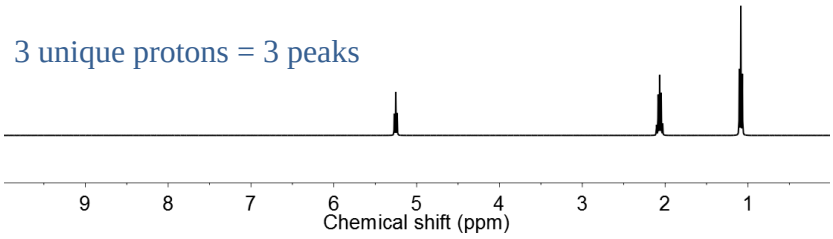
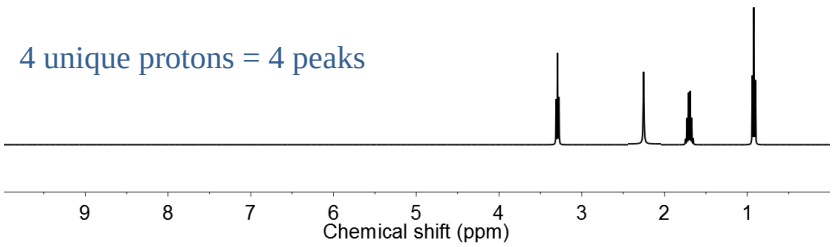


3 unique protons = 3 peaks



or

4 unique protons = 4 peaks



f. (5 points) What information can we extract from the splitting pattern of a peak?
Peak splitting provides information on how many hydrogen neighbors exist for a particular hydrogen (or group of equivalent hydrogens). In general, an NMR peak will be split into N + 1 peaks where N = number of hydrogens on the adjacent atom or atoms.

3. **Sample Preparation in Protein NMR** (30 points)

a. (15 points) What types of protein samples are good for NMR? Please comment on stability, concentration, expression conditions, and anything else you find relevant. Please refer to this website for more information: http://www2.chemistry.msu.edu/facilities/nmr/900mhz/MCSB_NMR_sample.html

i. Must be stable for several weeks at RT in the NMR tube.
ii. 0.1-2.5 mM concentration required.
iii. Have to be able to express it in E coli with minimal media that has been isotopically spiked.

b. (5 points) Why can't you run an NMR experiment on a single copy of your target molecule or protein?

i. You won't get enough signal from a single nuclear magnetic moment.
ii. You need several nuclei to align with the magnetic field so that you get a net stable longitudinal magnetic moment that you can measure.
iii. So if you have more nuclei in your sample, you have a higher chance of observing a signal – population statistics.

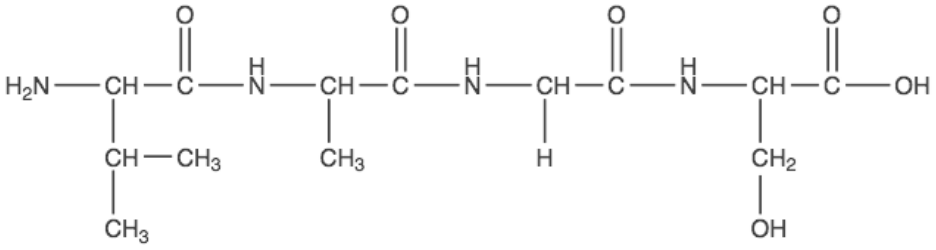
c. (5 points) Why is there a size limit in NMR?

i. Larger proteins have too many overlapping signals so it's hard to conclusively identify residues
ii. Larger proteins have slower tumbling rates

d. (5 points) The NMR spectrum of a peptide in its folded state is different than the spectrum of its unfolded state. How and why are the spectra different?

i. The spectra will not be identical because the amino acids will experience different local environments.
ii. Things like solvation, hydrogen bonding, interactions with neighboring residues, etc. can play into these observations.

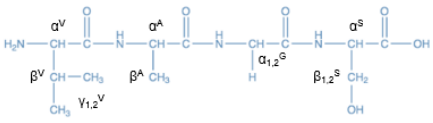
4. **Predicting 2D COSY Data** (45 points)
   You have the following four peptide sequence: Val-Ala-Gly-Ser.



   a. (30 points) Sketch the 2D-COSY spectrum on the attached grid, if the peptide were unstructured. Use the table provided at the end of the assignment as a guide.

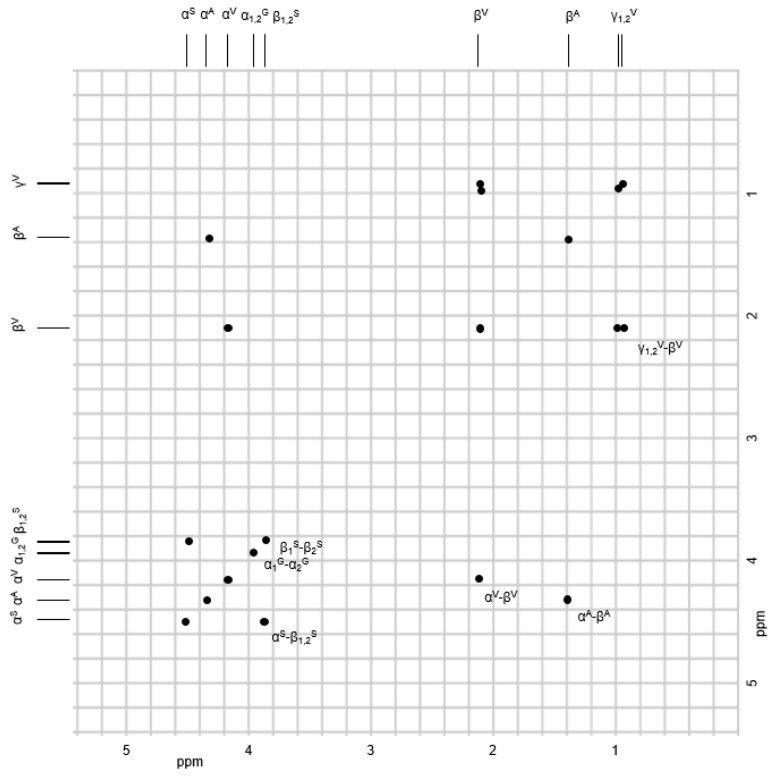
   Note: You can assume amine, amide, and hydroxyl protons will exchange with the deuterated solvent, and thus <u>will not be observed</u>. I can explain this further at office hours if you want to know why!

   If you're interested in hydrogen-deuterium exchange, you can read more about it here: https://en.wikipedia.org/wiki/Hydrogen%E2%80%93deuterium_exchange



Assume amine, amide, and hydroxyl protons solvent-exchange with deuterated solvent (NMR invisible).

Residue	NH	$C_\alpha H$	$C_\beta H$	Others
Gly	8.39	3.97		
Ala	8.25	4.35	1.39	$C_\gamma H_3$
Val	8.44	4.18	2.13	0.97, 0.94
Ser	8.38	4.50	3.88, 3.88	



b. (15 points) Assuming we completed this first step – that is, we assigned spin systems to specific amino acids in the sequence and peaks to specific protons – what are 3 kinds of structural information that we would next want to determine?
   i. distances between certain protons (<5 A)
   ii. which protons are involved in hydrogen bonding (through space NOE coupling)
   iii. dihedral angles (through bond J coupling)