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?

   b. (5 points) What happens to a NMR-active atom in an applied magnetic field?

   c. (5 points) Why is a radiofrequency pulse used in an NMR experiment?

   d. (5 points) What is the free induction decay (FID)?

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

   f. (5 points) Why does the FID decay?

   g. (5 points) How is a Fourier transform used in NMR?

   h. (5 points) What do different frequencies in this spectrum correspond to?

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)?

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

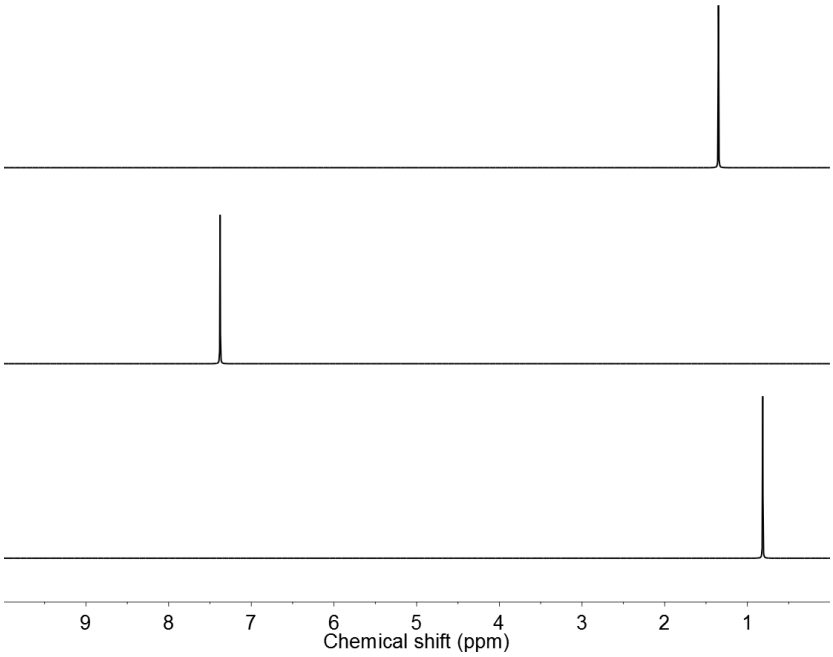
   c. (5 points) Given this, what frequency would you observe for the peak in part ‘a’ relative to the reference if the spectrum was recorded on a 300 MHz spectrometer?

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.

neopentane

cyclohexane

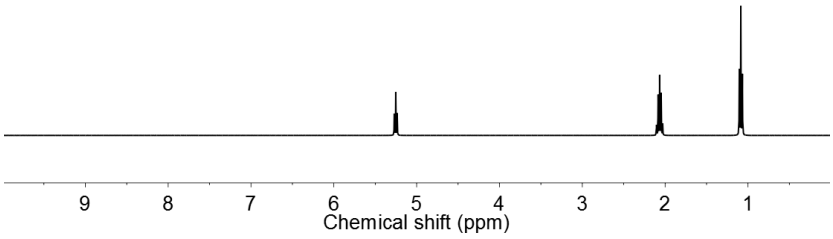
benzene



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.

or



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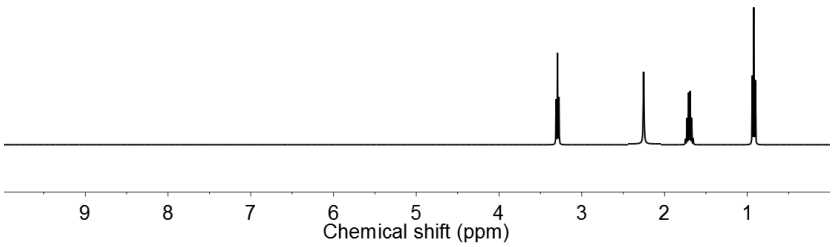


f. (5 points) What information can we extract from the splitting pattern of a peak?

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

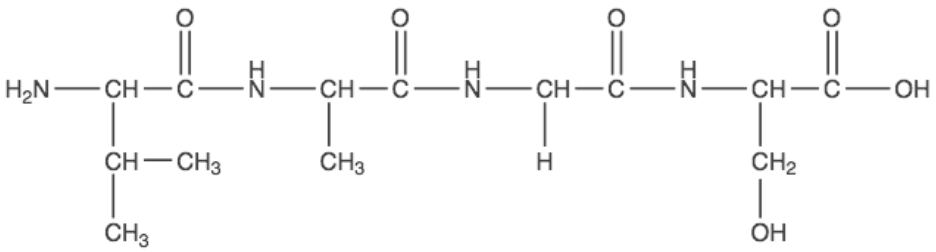
   b. (5 points) Why can't you run an NMR experiment on a single copy of your target molecule or protein?

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

   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?

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 will not be observed. 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

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?

^{1}H Chemical Shifts for the 20 Common Amino Acids (in ppm)

Residue	NH	$C_\alpha H$	$C_\beta H$	Others	
Gly	8.39	3.97			
Ala	8.25	4.35	1.39		
Val	8.44	4.18	2.13	$C_\gamma H_3$	0.97, 0.94
Ile	8.19	4.23	1.90	$C_\gamma H_2$	1.48, 1.19
				$C_\gamma H_3$	0.95
				$C_\delta H_3$	0.89
Leu	8.42	4.38	1.65, 1.65	$C_\gamma H$	1.64
				$C_\delta H_3$	0.94, 0.90
Pro(*trans*)		4.44	2.28, 2.02	$C_\gamma H_2$	2.03, 2.03
				$C_\delta H_2$	3.68, 3.65
Ser	8.38	4.50	3.88, 3.88		
Thr	8.24	4.35	4.22	$C_\gamma H_3$	1.23
Met	8.42	4.52	2.15, 2.01	$C_\gamma H_2$	2.64, 2.64
				$C_\varepsilon H_3$	2.13
Cys	8.31	4.69	3.28, 2.96		
Asp	8.41	4.76	2.84, 2.75		
Asn	8.75	4.75	2.83, 2.75	$N_\gamma H_2$	7.59, 6.91
Glu	8.37	4.29	2.09, 1.97	$C_\gamma H_2$	2.31, 2.28
Gln	8.41	4.37	2.13, 2.01	$C_\gamma H_2$	2.38, 2.38
				$N_\delta H_2$	6.87, 7.59
Lys	8.41	4.36	1.85, 1.76	$C_\gamma H_2$	1.45, 1.45
				$C_\delta H_2$	1.70, 1.70
				$C_\varepsilon H_2$	3.02, 3.02
				$N_\varepsilon H_3$	7.52
Arg	8.27	4.38	1.89, 1.79	$C_\gamma H_2$	1.70, 1.70
				$C_\delta H_2$	3.32, 3.32
				$N_\delta H$	7.17, 6.62
His	8.41	4.63	3.26, 3.20	$C_2 H$	8.12
				$C_4 H$	7.14
Phe	8.23	4.66	3.22, 2.99	$C_2 H, C_6 H$	7.30
				$C_3 H, C_5 H$	7.39
				$C_4 H$	7.34
Tyr	8.18	4.60	3.13, 2.92	$C_2 H, C_6 H$	7.15
				$C_3 H, C_5 H$	6.86
Trp	8.09	4.70	3.32, 3.19	$C_2 H$	7.24
				$C_4 H$	7.65
				$C_5 H$	7.17
				$C_6 H$	7.24
				$C_7 H$	7.50
				NH	10.22

Source: Adapted from data in K. Wüthrich (1986).