BMB/Bi/Ch 173 – Winter 2018

Homework Set 8.1 (100 Points) – Assigned 2-27-18, due 3-6-18 by 10:30 a.m.

TA: Rachael Kuintzle. Office hours: SFL 220, Friday 3/2 4-5pm and SFL 229, Monday 3/5 4-5:30pm.

1. **NMR Basics (30 points)**

   a. (4 points) What makes a nucleus NMR active?

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

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

   d. (2 points) What is the free induction decay (FID)?

   e. (2 points) Why does the FID oscillate up and down?

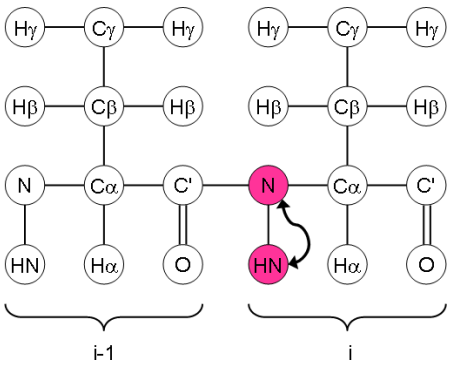
   f. (2 points) Why does the FID decay?

   g. (5 points) How is a Fourier transform used in NMR? What do different frequencies correspond to?

   h. (5 points) Define "chemical shift" in your own words. Incorporate the concept of "shielding" and describe how it is related to the magnetic field perceived by an NMR-active nucleus.

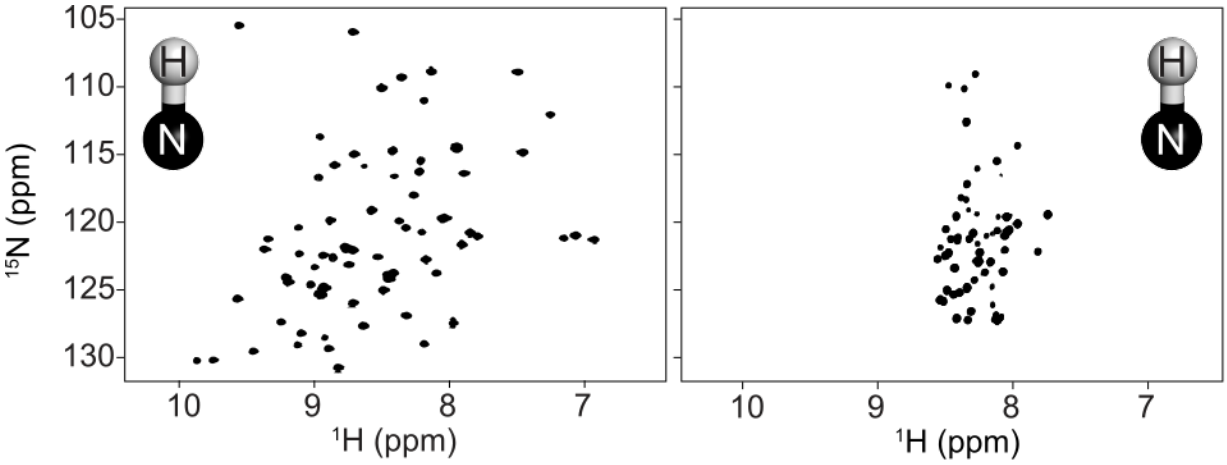
2) ^{15}N-HSQC (Heteronuclear Single Quantum Correlation) (28 Points)

A. (5 points) ^{15}N-HSQC is a type of 2D NMR experiment that measures ^{1}H-^{15}N one-bond coupling. The second dimension, which arises from transfer of magnetization from the proton to the NMR-active nitrogen isotope, spreads peaks out and makes them easier to assign. It requires only a small amount of ^{15}N-labeled material (less than 2 mg for a 20 kDa protein) and only ~30 minutes of spectrometer time. It yields a signal for almost every amino acid in a protein. For these reasons, a ^{15}N-HSQC spectrum is often the first NMR spectrum recorded on a new protein under investigation. An example of a nuclei pair which would give rise to an ^{15}N-HSQC peak is highlighted on the dipeptide below:

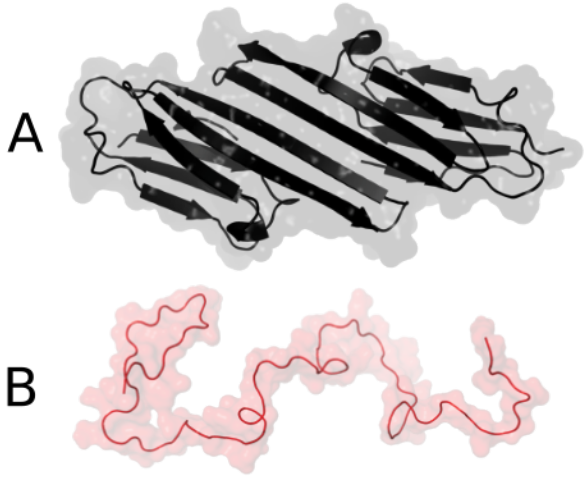


Which amino acid(s) doesn't (don't) have ^{15}N-HSQC signals? Explain why.

B. (10 points) Below are two ^{15}N-HSQC spectra of the same protein. Label each spectrum with the letter corresponding to the protein conformation it represents. **Explain your reasoning.**



Protein structure



C. (3 points) In NMR, T2 relaxation time is roughly proportional to the "tumbling rate," or rotational diffusion rate, of a molecule in solution. Quicker T2 relaxation times yield broad NMR peaks and poor signal-to-noise ratios. One of the fundamental limitations of NMR is the size of the molecule under study, in part due to the fact that larger molecules tumble more slowly and yield broad peaks which overlap too much. Assuming you already know the size of your protein, what is one thing you can learn about your protein sample from the broadness of your ^{15}N-HSQC peaks?

D. It is estimated that 40% of proteins have disordered regions of 40 residues or more (https://www.ncbi.nlm.nih.gov/pubmed/12368089).

   a. (2 points) Why is it difficult to assign resonances for disordered regions?

   b. (5 points) One approach to solve the problem above is to tag the protein with a paramagnetic tag. For example, a DOTA-M8 ytterbium tag (Yb-M8) (DOTA=1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) could be attached to a cysteine in the

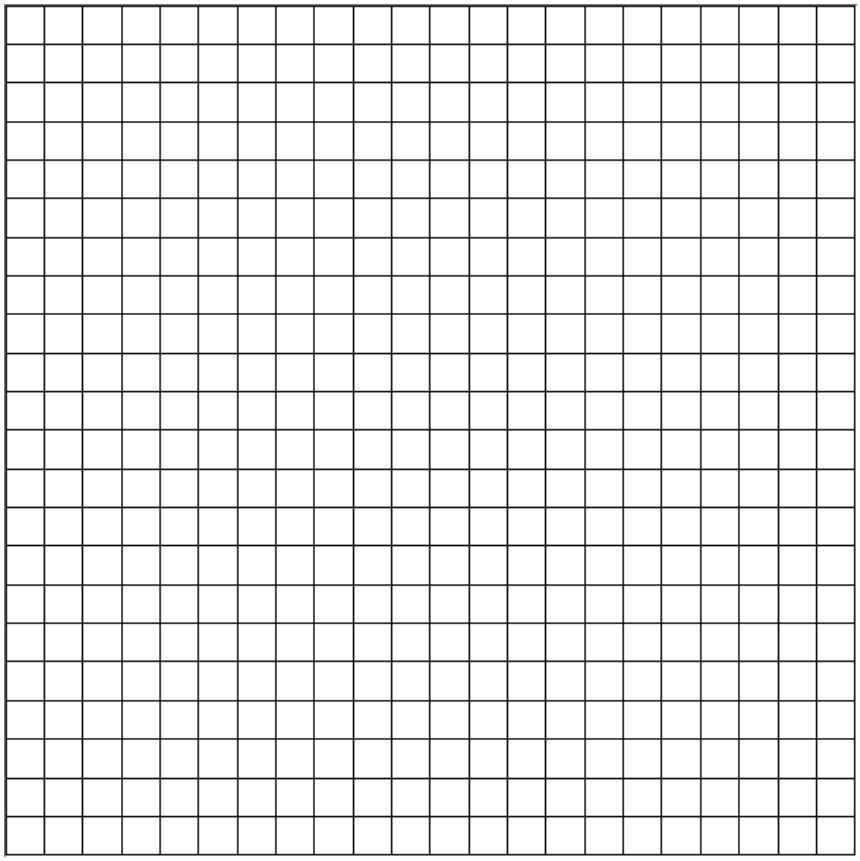
protein. Briefly, how does this enable ambiguous signals to be assigned to specific N-H groups in an HSQC spectrum?

c. (3 points) Why is it important to choose a tag that will not spontaneously isomerize?

3) Predicting 2D COSY data (20 points)

A. (30 points) You have the following four peptide sequence: Val-Ala-Gly-Ser

Sketch the 2D-COSY spectrum, if it was in the linear form. Use the table provided at the end of the assignment as a guide. Assume you are using a deuterated solvent; therefore, amine and hydroxyl protons will likely be replaced by deuterium and will not be observed.



4) Other NMR experiments (10 points)

A. (5 points) What type of magnetization transfer discussed in class can give you information about secondary structures present in your protein? **How** can you learn about your protein's secondary (and tertiary) structure from a NOESY experiment?

B. (5 points) Another technique you could use to investigate the tertiary structure of your protein is hydrogen-deuterium exchange (https://en.wikipedia.org/wiki/Hydrogen%E2%80%93deuterium_exchange). How does this experiment give information about protein tertiary structure?

5) Comparing NMR and X-ray crystallography (12 points)

A. (4 points) We can determine 3D molecular structure with both X-ray crystallography and NMR, but the process required to build a model from the data is quite different for each method. Briefly (in 1-2 sentences) describe the process of model-building for each method. (For X-ray crystallography, assume we already have phases.)

B. (4 points) Describe 2 technical limitations of NMR and X-ray crystallography (2 for each technique).

C. (4 points) Describe a protein structure that would be better to solve with NMR, and one that would be better to solve with X-ray crystallography. Explain your reasoning.

^{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
				$C_\epsilon H_3$	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
				$C_\epsilon H_2$	3.02, 3.02
				$N_\epsilon H_3$	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).