Homework for BCH 581 – NMR spectroscopy

Email your answers to klara.briknarova@umontana.edu by 10:30 am on Monday March 1.

If you write some parts by hand and scan them, please make sure your writing is legible! Show your work and explain your reasoning. Be concise.

1. Which techniques can be used to determine the 3-dimensional structures of biological molecules? List at least one advantage and one limitation for each method.

2. An NMR spectrometer operates at 1H Larmor frequency of 600.13 MHz.

(a) Calculate the Larmor frequency of 15N.

(b) Calculate the magnetic field.

3. On the NMR spectrometer described in question 2, the observed splitting of a 1H backbone amide signal due to one-bond J-coupling to the 15N atom is 0.153 ppm.

(a) Calculate the value of the J-coupling in Hz.

(b) Calculate the value of the splitting, in both Hz and ppm, that is observed for the signal from the 15N atom.

4. In a 2D 1H-15N HSQC spectrum that was acquired on the NMR spectrometer described in question 2, a signal from backbone amide is observed with a 1H chemical shift of 9.023 ppm and 15N shift of 123.87 ppm.

Calculate the 1H and 15N chemical shifts of this signal in a 1H-15N HSQC spectrum that was acquired on an NMR spectrometer that operates with 1H Larmor frequency of 800.13 MHz.

5. Explain what a 90º pulse is and what it does.

6. Provide two reasons for why the absorption line shape is preferred over the dispersion line shape.

7. Explain what effect fast transverse relaxation will have on NMR spectra.

8. Analysis of magnetization during 2D NOESY experiment:

(a) When analyzing magnetization during 2D NOESY, I suggested (on p. 15 of the NMR #3 notes) that we ignore the My term that contains $sin⁡(Ωt\_{1})$ after point D. Show what will become of to this term after the mixing time ($t\_{mix}$) and the last 90º pulse if you do not ignore it, i.e. derive equations for Mx, My and Mz at point F that originate from this term. Use the right handed rotation convention and ignore transfer of magnetization to other spins during the mixing time ($t\_{mix}$).

(b) Consider the same 2D NOESY pulse sequence as shown on p. 13-16 of the NMR #3 notes, but with the phase of all three 90º pulses set to **–y** (i.e. **negative y**). Show the magnetization that you will have at the beginning of the FID, i.e. derive equations for Mx, My and Mz at point F. Ignore transfer of magnetization to other spins during the mixing time ($t\_{mix}$) but do not ignore any other terms along the way. Can you imagine any use for the data acquired in this way?

(c) Consider the same 2D NOESY pulse sequence as shown on p. 13-16 of the NMR #3 notes, but with the phase of the first 90º pulse set to **x**, the phase of the second 90º pulse set to **y**, and the phase of the third 90º pulse set to -**y** (i.e. **negative y**). Show the magnetization that you will have at the beginning of the FID, i.e. derive equations for Mx, My and Mz at point F. Ignore transfer of magnetization to other spins during the mixing time ($t\_{mix}$) but do not ignore any other terms along the way. Can you imagine any use for the data acquired in this way?

9. What is the main difference between COSY and NOESY spectrum? What are these experiments used for?

10. Prepare a sketch of a COSY spectrum for a tryptophan residue that is part of a larger polypeptide. Assume that the chemical shifts of the atoms are close to their random coil values.

**Use the data from the attached table** (from Wüthrich, K., 1986, NMR of Proteins and Nucleic Acids, John Wiley and Sons)**, which contains chemical shift for one more atom than the table provided in class.**

11. For a particular backbone amide signal in a 2D 1H-15N HSQC spectrum, three peaks were observed in an HNCACB spectrum with 13C chemical shifts of 64.0 ppm, 58.5 ppm, and 45.0 ppm, and one peak was observed in a CBCACONH spectrum with 13C chemical shift of 45.0 ppm. To which residue type (i.e. amino acid) does this amide signal most likely belong? What is the most likely identity of the preceding residue, i.e. of the amino acid that is N-terminal of this residue? What is the most likely identity of the following residue, i.e. of the amino acid that is C-terminal of this residue? Can you say anything about the secondary structure that this residue is part of?

A note: CBCACONH and CBCA(CO)NH are two slightly different names for the same experiment. In addition, HNCOCACB (shown in some of the slides in nmr #4 notes) will yield signals in the same locations and so can be considered identical for our purposes.



