Methods in Biophysical Chemistry – CH 8990 03
Assignment 6

Due Monday, April 14

1. The typical $^{1}J_{NH}$ splitting in an amide group is 92 Hz (actually, it's -92 Hz because of the negative γ for ^{15}N).

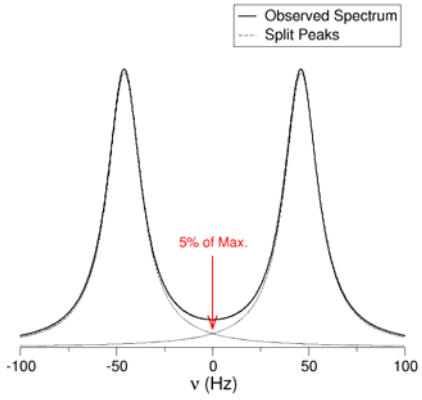
   a. How many ppm would this correspond to for protons at 600 MHz? At 800 MHz? (3 points)

   b. As discussed in class, the Fourier transform of an exponential decay is a Lorentzian line shape. Provided that the signal decays with a time constant T_2, i.e. ($f(t) = e^{-t/T_2}$), the functional form of a Lorentzian in the frequency domain (ω in rad s^{-1}) is:

$$F(\omega) = \frac{CT_2}{1 + (T_2\omega)^2}$$

   Where C is a constant scaling factor. If T_2 is short, the peak will be broad. If T_2 is long, the width will be narrow. When measuring $^{1}J_{NH}$ coupling constants, it's best to have as much resolution as possible between the split peaks. Resolution depends on the coupling constant itself as well as the line width in the transformed spectrum. A well-resolved splitting will have decayed to 5% or less of its maximum height at ± ½ $^{1}J_{NH}$ (see below). What would T_2 have to be in order for the splitting to be resolved at this level? You may assume that the line shape is Lorentzian and the line width depends only on the amide proton T_2.

   The figure below is designed to help you visualize the problem, and you can use it to check your answer. However, you will not receive full credit if you solve the problem graphically. (6 points)



   c. Using your value from above, estimate the largest protein by which you could measure couplings using this method for ^{1}H and ^{15}N spins. *Hint:* Your slides contain a table of T_2 vs. τ_c, and you know that 2.5 kDa ≈ 1 ns for τ_c. (5 points)

d. Bax and workers have argued that the uncertainty (in Hz) in a peak position is given by this formula:

$$\sigma = \frac{FWHM}{2Q}$$

In this formula, FWHM is the full width at half maximum of a peak, and Q is the signal to noise measured for that peak.

Assume that the FWHM for each peak in an amide proton splitting is 1 Hz. Both peaks have a signal to noise ratio of 30. What is the uncertainty in the coupling if you measure $^1J_{NH}$ by taking the difference of the peak maxima? Remember this is a difference of *two* peak positions! (3 points)

2. Sketch the Fourier transforms of the following functions. Rough sketches are fine. (6 points)

a. $f(t) = [\cos(\omega_1 t) - i\sin(\omega_1 t)] + [\cos(\omega_2 t) + i\sin(\omega_2 t)]$
b. $f(t) = [\sin(\omega_0 t)]e^{-t/T_2}$

3. If the Fourier transform of $f(t)$ is $F(\omega)$ and the Fourier transform of $g(t)$ is $G(\omega)$, prove that the Fourier transform of $f(t) + g(t)$ is $F(\omega) + G(\omega)$. (4 points)

4. On a 600 MHz (proton) magnet, what is the frequency difference between two ^{13}C resonances that differ by 5 ppm? What about for two ^{15}N resonances? (5 points)

5. The following table gives chemical shifts for a series of residues in a protein:

Residue Number	Name	H_N	N	C_α
11	Val	9	100	60
12	Ala	8	110	50
13	Cys	10	105	55
14	Tyr	7	120	58
15	Asn	8	100	53

The HNCA is a three dimensional experiment that shows correlations between amide nitrogen frequencies (t_1), C_α frequencies (t_2) and H_N frequencies (t_3). In this experiment, magnetization is transferred from the nitrogen to the C_α of the same residue *and* the C_α of the previous residue. This is followed by transfer back to the same amide nitrogen, and then to the amide proton for detection.

Sketch the following 2-dimensional planes from the three-dimensional spectrum using the chemical shift values above: (i) ^{15}N ppm = 110 (C_α-H_N plane), (ii) ^{15}N ppm = 105 (C_α-H_N plane), and (iii) ^{13}C ppm = 55 (N-H_N plane). (10 points)

6. Consider an Alanine residue in a polypeptide chain. Assume that the polypeptide has been labeled with ^{13}C and ^{15}N, and that the pH is low enough that amide proton exchange is negligible.

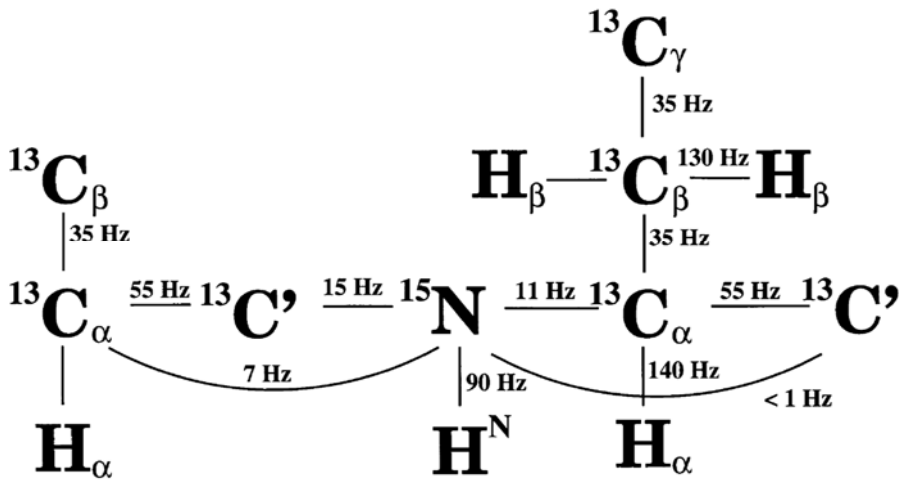
   a. Draw the 1-D spectra for ^{1}H, ^{15}N, and ^{13}C for this Ala residue. You do not have to consider scalar couplings, but the chemical shift values you use should be reasonable (i.e., within the range expected for such spins). (5 points)

   b. Consider the H_α resonance for Ala, which has the following active couplings:

Coupling	# of Coupled Nuclei	Value (Hz)
$^{1}J_{C\alpha H\alpha}$	1	140
$^{3}J_{H\beta H\alpha}$	3	10
$^{3}J_{HNH\alpha}$	1	3

   Assuming that it is possible to resolve every coupling, draw the expected proton spectrum for this system. Center your spectrum on 4 ppm. (7 points)

   c. Draw a close-up of the C_α resonance. For this nucleus, it is safe to assume that the only significant couplings are those shown in the diagram below. Be sure to indicate which couplings you are considering. (8 points)



Taken from Sattler, M. *et al*. (1999) "Heteronuclear multidimensional NMR experiments for the structure determination of proteins in solutions employing pulsed field gradients." *Prog. NMR Spectroscopy*. **34** (2): 93-158.

For this problem, you may find it convenient to write a simple computer program to generate the spectrum. If you choose to do this, your final result should be a sum of sharp Lorentzian peaks (T_2 > 300 ms), each peak centered at the appropriate frequency. You may assume that C_α resonance is centered on 0 Hz. Then, couplings will give rise to symmetric doublets about 0 Hz. At the very least, you should start with a table of all of the active couplings so you can keep track of the splittings.