Biology 5325

Protein Structure & Function

Examination #2

NMR Spectroscopy
X-Ray Crystallography

December 20, 2005

1. (10 points) Define the following with words (and equations, where appropriate):

μ_m

B_o

ω_o

B_1

resonance
(in NMR)

2. You work for GPF (Gigantic Pharmaceutical Firm, Inc.) and have been asked to characterize a novel pleckstrin homology domain by NMR. This domain constitutes the N-terminal 124 residues of a large, multi-domain cytoplasmic protein called Ftk (Fred's tyrosine kinase). It specifically binds the phospholipid PI(3,4,5)P_3 and its soluble inositol phosphate metabolite I(1,3,4,5)P_4. Point mutations in the PH domain of Ftk have been linked to diabetes, but the mechanism is unknown. Your colleagues have expressed the recombinant PH domain of Ftk in bacteria and purified stable forms of the wild-type and mutant proteins. Although the domain has not yielded diffraction-quality crystals, the 1:1 complex with I(3,4,5)P_3 is soluble to ~1 mM in aqueous buffer at pH 7. You have been asked you to solve the three-dimensional structure of the wild-type complex by NMR. Devise a suitable NMR strategy and answer the following questions:

a. (4 points) Which isotope-enrichment scheme, if any, would you use to study this ligand-protein complex?

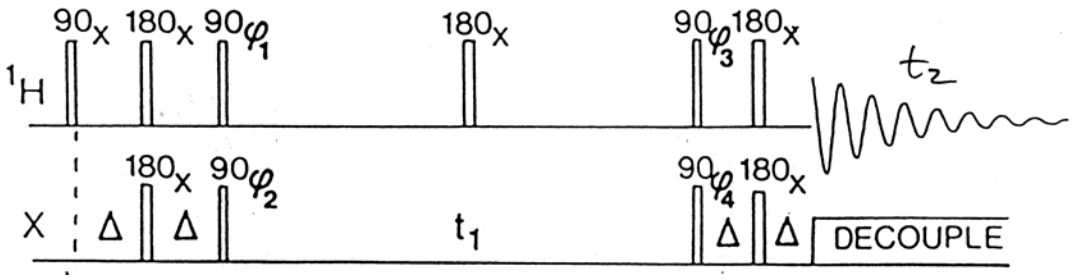
b. (8 points) Which NMR experiments might you use to establish resonance assignments for the protein backbone atoms? In your answer, list the name of each experiment and the atomic correlations established

by it. Please sketch a protein backbone segment and map the correlations onto that segment. Indicate how the experiments are used together to establish the assignments.

c. (4 points) Describe one approach you could use to map the protein's secondary structure. In your answer, list the NMR parameters you would measure, the type of information obtained from them and the manner in which this information would be used to define the secondary structure.

d. (4 points) Describe one approach that you could use to characterize the interactions between the PH domain and I(1,3,4,5)P_4. How might you locate the ligand binding site?

3. The pulse sequence for the ^{1}H-^{15}N HSQC experiment is shown below, where X=^{15}N:



a. (8 points) Identify four basic components or building blocks of the HSQC pulse sequence.

b. (8 points) Using the product operator formalism, describe the state of the ^{1}H-^{15}N (IS) spin system immediately after the second set of 90º pulses, at the beginning of the t_1 period. Include in your answer the operations involved for each pulse and delay period. You may assume that the IS spin system represents proton-nitrogen pairs from the backbone amide positions of a uniformly ^{15}N-enriched protein. The Δ delays are fixed at $1/(4J_{NH})$ seconds. For the second set of 90º pulses,

φ_1 = y and φ_2 = x. You may neglect chemical shift evolution during the delays. Helpful hint: $\cos^2(\pi/4) = \cos(\pi/4)\sin(\pi/4) = \sin^2(\pi/4) = 0.5$.

c. (4 points) Name two ways in which HSQC can be used in NMR studies of proteins.

4. (10 Points) Protein X crystallizes under the following conditions:

Polyethyleneglycol (PEG, MW4000) = 14%
Tris, pH8, 0.1M
$CaCl_2$, 0.1M

The reciprocal lattice dimensions were obtained from x-ray diffraction analysis and found to be a*=0.01Å^{-1}, b*=0.015Å^{-1}, c*=0.0066Å^{-1}, α*=90, β*=90 and γ*=90. Determine the real space lattice dimensions and the name of the crystal system.

5. (10 Points) Protein X crystals diffract to a resolution of 1.7Å. Determine the number of reflections, N, that should be measured to obtain such a data set. The value of N is equal to the number of reciprocal lattice points contained in the volume of the sphere of reflections. Recall that the sphere of reflections is defined in reciprocal space, and therefore its "volume" has units of 1/Å^3. So, in reciprocal space we have $N = (4/3)\pi|S|^3/V^{-1}$, or in real space, $N = (4/3)\pi V/d^3$.

6. (10 Points) The diffraction pattern of protein X demonstrates systematic absences along the a* axis consistent with the $P2_122$ space group. Describe the type of systematic absences that are expected. Using the structure factor equation, give a demonstration of these systematic absences. Note that $\mathbf{F}(hkl) = \sum_{j=1}^{N} f_j(hkl)e^{i\phi_j(hkl)} = \sum_{j=1}^{N} f_j(hkl)e^{2\pi i[hX+kY+lZ]}$.

7. (10 Points) Data taken from protein X crystals soaked in uranium acetate at a concentration of 1 mM for 24 hours show a 20% difference relative to data taken on the native crystals. A Patterson map calculated using the formula $|F_H| = |F_{PH}| - |F_P|$ gives peaks at:

peak 1:	u = -0.5	v = 0.3	w = 0.4
peak 2:	u = 0.2	v = 0.3	w = 0.0
peak 3:	u = -0.3	v = 0.0	w = 0.4

The $P2_122$ space group has symmetry operators ("equivalent positions") as follows:

(x,y,z), (-x,-y,z), (½+x,-y,-z), (½-x,y,-z)

Determine the position of the heavy atom(s) in the unit cell. Explain how the α_p (phase for the protein) can then be calculated.

8. (10 Points) The use of two or more heavy metal derivatives and difference Patterson maps to find a set of initial phases is referred to as the multiple isomorphous replacement (MIR) method. Briefly describe two other ways to determine an initial set of protein phases, and discuss the applicability and advantages of these alternative methods.

PRODUCT OPERATORS FOR A WEAKLY COUPLED IS SPIN SYSTEM

	Ix	**Iy**	**Iz**	**Sx**	**Sy**	**Sz**	**2IzSz**
Ix	E/2	-Iz	Iy	E/2	E/2	E/2	2IySz
Iy	Iz	E/2	-Ix	E/2	E/2	E/2	-2IxSz
Iz	-Iy	Ix	E/2	E/2	E/2	E/2	E/2
Sx	E/2	E/2	E/2	E/2	-Sz	Sy	2IzSy
Sy	E/2	E/2	E/2	Sz	E/2	-Sx	-2IzSx
Sz	E/2	E/2	E/2	-Sy	Sx	E/2	E/2
2IzSz	-2IySz	2IxSz	E/2	-2IzSy	2IzSx	E/2	E/2
2IxSz	E/2	-2IzSz	2IySz	-2IxSy	2IxSx	E/2	Iy
2IySz	2IzSz	E/2	-2IxSz	-2IySy	2IySx	E/2	-Ix
2IzSx	-2IySx	2IxSx	E/2	E/2	-2IzSz	2IzSy	Sy
2IzSy	-2IySy	2IxSy	E/2	2IzSz	E/2	-2IzSx	-Sx
2IxSx	E/2	-2IzSx	2IySx	E/2	-2IxSz	2IxSy	E/2
2IxSy	E/2	-2IzSy	2IySy	2IxSz	E/2	-2IxSx	E/2
2IySx	2IzSx	E/2	-2IxSx	E/2	-2IySz	2IySy	E/2
2IySy	2IzSy	E/2	-2IxSy	2IySz	E/2	-2IySx	E/2