

Biology 5357

Chemistry & Physics of Biological Molecules

Exam #1 Answer Key

1. The paper describing this work from Alan Fersht's group is *Folding and Design*, 2, 363-368 (1997). The correct answers for part (a) are given below and in Table 5 of the paper. Remember that DDG is 0 at the midpoint denaturant concentration. Then for the A16G mutant, the $\Delta\Delta G$ at 3M GdmCl is $1.80 \times (3.0 - 3.44) = -0.79$ kcal/mol, and similar for other proteins. The values relative to wild type are then obtained by subtracting the WT $\Delta\Delta G$ of -1.90 from the value for each mutant.

Protein Sequence	m_{U-N} (kcal/mol/M)	[GdmCl] _{50%} (M)	$\Delta\Delta G_{U-N}^{3M}$ (kcal/mol)	Relative to WT (kcal/mol)
Wild-type	1.90	4.00	-1.90	-0-
A16G	1.80	3.44	-0.79	+1.11
I57A	1.93	1.79	+2.34	+4.24
A16G/I57A	2.45	0.79	+5.42	+7.32
I57V	1.82	4.10	-2.00	-0.10

For part (b), we see that the A16G mutation destabilizes the protein relative to WT by 1.11 kcal/mol, and the I57A mutant is destabilized by 4.24 kcal. But the double mutant is destabilized by 7.32 kcal/mol, which is about 2 kcal more than the sum of the individual single mutants. This indicates that the interaction of A16 with I57 in the WT protein is net destabilizing relative to the smaller mutant side chains (G16 and A57). We conclude that the WT protein is actually overpacked and contains (small) unfavorable steric interactions – a somewhat unusual situation. This is confirmed in part (c) by the fact that the I57V single mutation stabilizes the protein by a small amount relative to WT. Again, the removal of a –CH₂– group in going from I to V must be reducing some steric crowding in this region of the structure.

2. a. The binding of a ligand is related to the difference between its interactions with the protein (in the bound form) and its interactions with water or solvent (in the unbound form). Thus favorable interactions with the protein do not always increase strength of binding. For example, a ligand group may

form a hydrogen bond to a protein. But if that same ligand group forms an equally strong bond to water in free solution, it will make little contribution to overall binding.

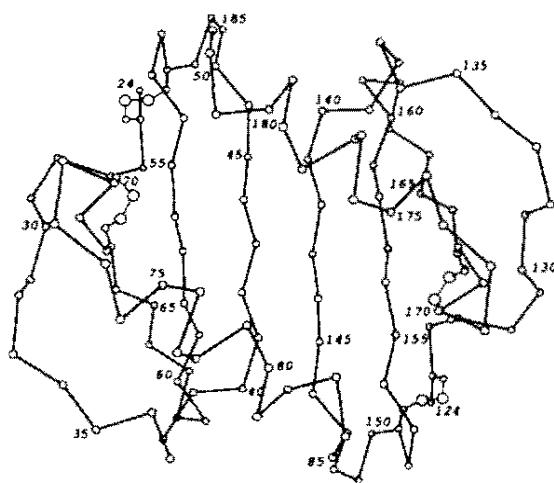
b. The hydrophobic effect does indeed make a negative contribution to the entropy of protein folding-unfolding transition, in agreement with the fact that transfer of small hydrocarbons from nonpolar solvents to water has a negative ΔS . But an even larger (and positive) contribution to the entropy of comes from the increased configurational entropy of the chain in the unfolded state.

c. Protein folding is a highly cooperative event resulting from the accumulation of many small interactions. A single hydrogen bond may not be highly favorable. But succeeding bonds will tend to be more favorable since the previous bonds reduce the entropic cost of making additional bonds in the folded form.

3. This question is taken from work published in Nature, 344, 363-364 (1990) and Nature, 348, 397 (1990). As stated in part (a), residue 26 is located in a turn region directed away from the rest of the structure. The mutant data shows that placing a more polar side chain at position 26 stabilized the protein, while the hydrophobic W26 mutant is destabilizing. This is explained by the fact that position 26 is apparently more exposed to solvent in the folded form of the protein than in the denatured/unfolded form. This is an atypical result, hence the publication in Nature (!). A full answer to part (b) requires analysis of the equilibrium unfolding curve, exactly as described in the Pace review article from the course web site. Full credit was given for simply choosing two points in the transition region, estimating the fractions folded and unfolded, followed by the equilibrium constant, and the resulting DG value. As noted in the Pace review and in class, the ΔC_p for unfolding is nonzero, typically about 12 cal/mol/K/residue. Thus, simple linear extrapolation of thermal unfolding data can lead to substantial errors.

4. Two points were given for the correct 2-dimensional structure/connectivity of Asp, and two points for Phe. An additional two points were given for having the correct L chirality of both amino acids (no points for one of each chirality!), and two points for the correct protonation state at the N-terminus and the Asp side chain.

5. The paper describing the original structure determination is *Journal of Biological Chemistry*, 264, 2092-2099 (1989), and the drawing below is taken from Figure 5. As you look down on the page, the strands of the sheet run vertically, and the two helices are from upper left to lower right. Given the canonical twist of sheets as discussed in class, the sheet will tend to curl below the page at the lower left and upper right, and curl above the page at the upper left and lower right corners. Thus the high ridges running diagonally across the sheet will be from upper left to lower right above the page, and from lower left to upper right below the page. Since helices tend to pack along these ridges, the helices should both lie above the plane of the page.



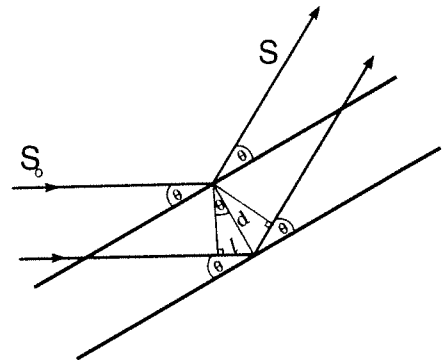
EXAM KEY

Bio5325 Midterm Exam, 2007
Ellenberger

1. (5 pts.) The diagram below shows a one-dimensional crystal with a spacing of $d = 10 \text{ \AA}$ between Bragg reflecting planes. Bragg's law states that $\lambda = 2d \sin\theta$. For x-rays of wavelength $\lambda = 1.5 \text{ \AA}$, at what 2θ angle (i.e., the angle of S relative to the direct beam S_0) would the diffracted waves add together in phase and be recorded on a detector placed at right angles to the incident radiation S_0 ? Show your work for full credit.

$$\frac{\lambda}{2d} = \sin\theta$$

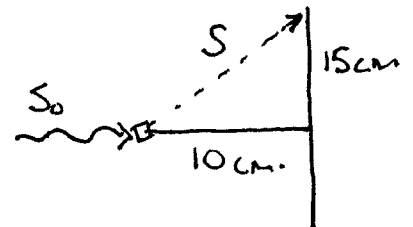
$$\theta = \sin^{-1}\left(\frac{\lambda}{2d}\right) = \sin^{-1}\left(\frac{1.5}{2 \cdot 10}\right) = 4.3^\circ$$



2. (5 pts.) For a 30 cm diameter detector centered on S_0 and placed 10 cm from the crystal, what is the minimum spacing d that could be recorded on the detector? Show your work.

$$2\theta_{\max} = \tan^{-1}\left(\frac{15}{10}\right) = 28.2^\circ$$

$$d = \frac{\lambda}{2\sin\theta} = \frac{1.5 \text{ \AA}}{2\sin(14.1)} = 1.59 \text{ \AA}$$

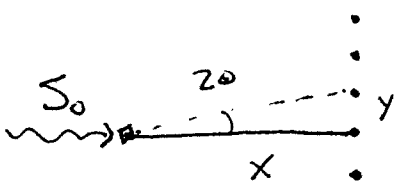


3. (5 pts.) The diffraction pattern from a crystal with unit cell dimensions $a = 30 \text{ \AA}$, $b = 60 \text{ \AA}$, and $c = 90 \text{ \AA}$ was recorded using x-rays of wavelength $\lambda = 1.5 \text{ \AA}$, and is shown below. In the central region the spacings between reflections are 2.5 mm in the horizontal direction and 3.75 mm in the vertical direction. The indexing program DENZO identifies the correct unit cell dimensions for our crystal and estimates the crystal to detector distance for this image. What is the correct value of the crystal to detector distance?

$$\sin\theta = \frac{\lambda}{2d}; \quad \theta = \sin^{-1}\left(\frac{\lambda}{2d}\right)$$

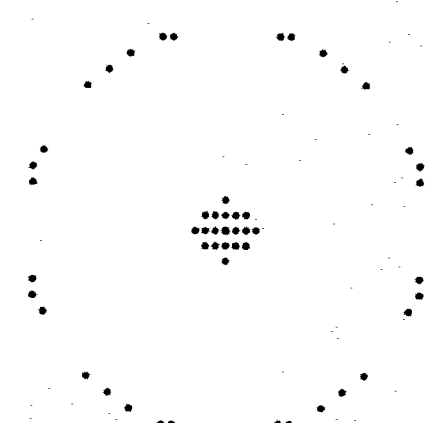
For $a = 30 \text{ \AA}$, $\theta = \sin^{-1}\left(\frac{1.5}{2 \cdot 30}\right) = 1.43^\circ$
 For $b = 60 \text{ \AA}$, $\theta = \sin^{-1}\left(\frac{1.5}{2 \cdot 60}\right) = 0.72^\circ$
 For $c = 90 \text{ \AA}$, $\theta = \sin^{-1}\left(\frac{1.5}{2 \cdot 90}\right) = 0.48^\circ$

$$\tan 2\theta = \frac{y}{x}; \quad x = \frac{y}{\tan 2\theta}$$



$$x = \frac{3.75 \text{ mm}}{\tan(2 \cdot 0.72)} = 149 \text{ mm}$$

$$x = \frac{2.5 \text{ mm}}{\tan(2 \cdot 0.48)} = 149 \text{ mm}$$

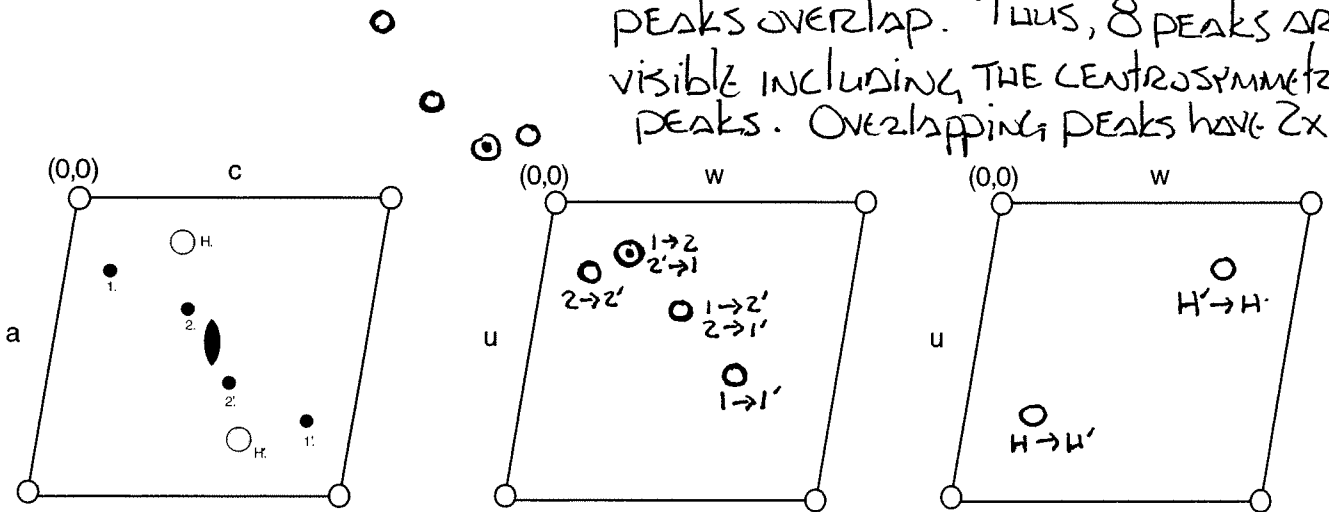


By TRIAL AND ERROR, WE FIND that the vertical spacings correspond to b^* and the horizontal spacings are c^* . Detector distance is 149 mm.

4. A molecule consisting of 2 atoms (blue spheres) packs in a two-dimensional crystal with a second molecule related to the first by a 2-fold rotation (black ellipse). A heavy atom derivative is prepared with one heavy atom (yellow sphere) associated with each molecule in the unit cell.

(4 pts) In the central diagram, draw the native Patterson function (without the heavy atom), using filled circles to show peaks corresponding to the interatomic vectors within one molecule and using open circles to show peaks relating pairs of atoms in two different molecules. How many native Patterson peaks are located in the unit cell?

12 peaks ($4^2 - 4 = 12$), but 2 pairs of peaks overlap. Thus, 8 peaks are visible including the centrosymmetric peaks. Overlapping peaks have 2x density.



(4 pts) In the diagram above on the right, draw the peaks corresponding to the heavy atom difference Patterson (recall that the coefficients of a difference Patterson are $(F_{PH} - F_P)^2$)

(2 pts) The symmetry operators of our two-dimensional crystal are (X, Z) and $(-X, -Z)$. What is the equation for the Harker vector?

$$U = X - (-X) = 2X \quad \text{OR} \quad -X - X = -2X$$

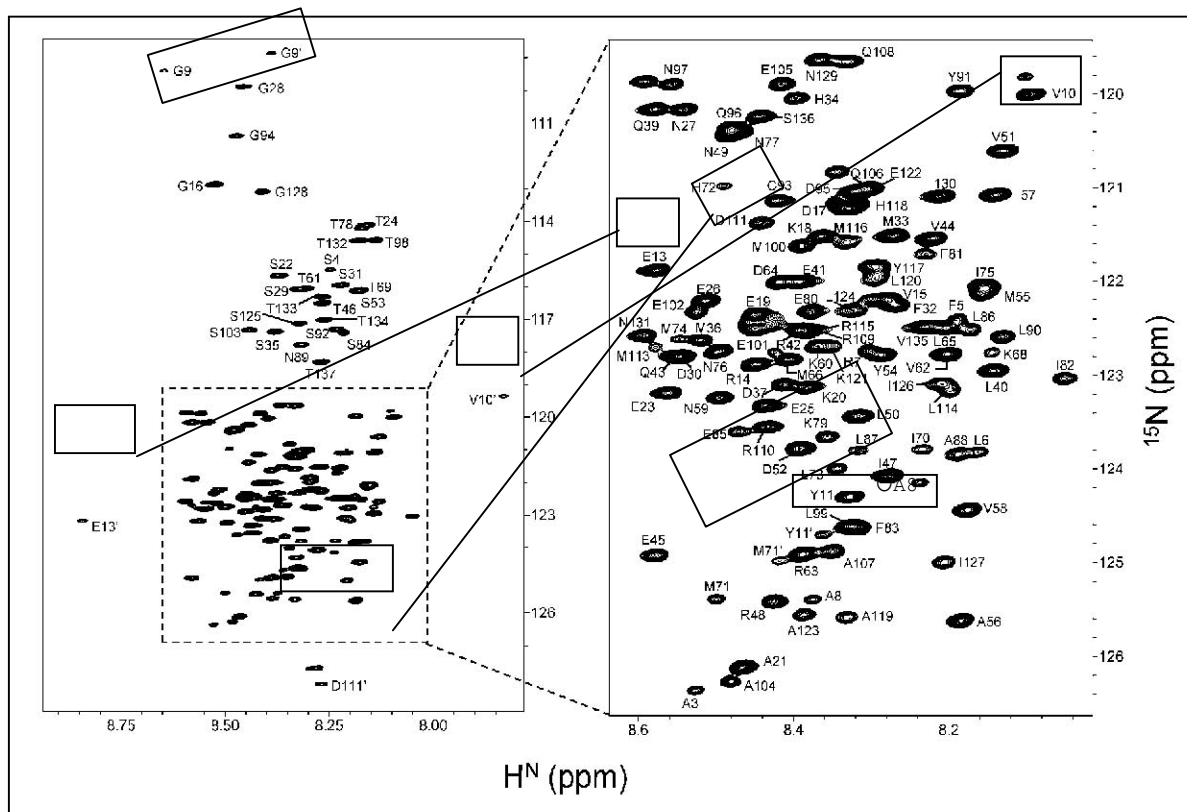
$$W = Z - (-Z) = 2Z \quad \quad \quad -Z - Z = -2Z$$

(2 pts) If one of the heavy atoms has real space coordinates of $X = 1/6$ and $Z = 1/3$, what are the coordinates (U, W) of the peak(s) in the difference Patterson?

$$(U, W) = (-1/3, -2/3) \quad \text{OR} \quad (1/3, 2/3)$$

5. (5 pts) List at least 2 factors that limit the accuracy of phase information obtained by heavy atom replacement (isomorphous replacement) methods.

NONISOMORPHISM OF THE HEAVY ATOM DERIVATIVE.
 INCOMPLETE OCCUPANCY OF HEAVY ATOM BINDING SITES.
 INACCURATE SCALING OF NATIVE, DERIVATIVE DATA.



Q1. What can you say about the structure of protein X from this HSQC? Upon what information do you base your opinion?

A. The protein is unstructured, since the chemical shift dispersion of the amide protons is very poor. Peaks are also broad in the ^1H dimension, suggesting that there are multiple structures in fast exchange. However, at least two segments of the protein are undergoing slow conformational exchange, since they have two amide peaks.

Q2. Locate G9/G9', D111/D111', V10/V10', E13/E13', M71/M71', Y11/Y11', and A8/A8' (Position of A8' is circled, as it is not visible at this contour threshold, i.e. it has low intensity).

a. Give two possible explanations for the appearance of these two sets of crosspeaks in protein X, taking into account their relative intensities and their chemical shifts.

A2a. Two elements of the protein are undergoing slow exchange on the NMR timescale, and the rate of their exchange can be calculated from the difference in their frequencies. The elements are from amino acid 8-11,13, and 71 and 111. Since the protein is predominantly unstructured, these regions aren't loops, since loops form when the protein folds. Instead, I would hypothesize that they come from a primary folding nucleus, such as a core or a hydrophobic turn.

The presence of two distinct resonances could be interpreted as indicative of two distinct conformations, or as evidence of multiple states that are averaged into the observed peaks. One resonance is located among the other amides resonances in the crowded region of the spectrum, but the other is shifted outside the bulk, suggesting that it represents a more

structured environment that gives it a unique chemical shift. The relative peak intensities show that the unstructured environment dominates in these conditions (pH 5 and 15°C).

If you squint, you can compare the linewidths of the resonances. Those from the unstructured conformation are rather broad; there could be multiple conformations present that average out to a broad chemical shift range. Alternatively, the protein is tumbling slowly in an extended conformation that slows its exchange and broadens the linewidths. The broad linewidth could indicate multiple conformations that are averaged, or a single conformation that is slowly exchanging.

Q2b. Now test your explanations, applying the method of your choice to the NMR sample. Predict the outcome of your experiments.

A2b. Experiments I would do to sort this out are to first raise the temperature. I would expect that those resonances that come from a putative 'structured' conformation would disappear, since this conformation is less populated (and presumably less stable). In contrast, if I lowered the temperature (say to 5° C) the exchange between conformations would be slowed, and the two resonances could have equal intensity.

At a lower temperature, it is possible that more resonances would show multiple chemical shifts, if a global conformational exchange rate between structured and unstructured species is slowed. (Realize also that at low temperatures, all peaks become broader since the tumbling of the molecule is slower). If there are two conformations of the protein, then I'd expect to see two peaks from more resonances, but if there are multiple conformations, then I'd look for multiple amide resonances. This is tricky because they are likely to be weak and broad.

$^1\text{H}/^{15}\text{N}$ HSQC experiments would be used to assess the exchange properties of the protein. Calculating the chemical shift differences between resonances will give the rate of exchange between conformations, and distinguish local *vs* global fluctuations.

The low pH of the solution slows the amide proton exchange with solvent protons, and if it's raised to 6-7, several scenarios are possible. First, the exchange rate between amide protons and water could be so rapid that the amide protons would disappear. That would be the end of the experiment. Alternatively, there could be a subset of amide resonances that disappear, leaving others that are more stable; those stable ones could come from a region of the protein that is more protected presumably due to some residual structure. It is also possible that the protein denatures at low pH, but becomes more structured at pH 7, so the entire spectrum could change in response to a global conformational change.