

**Biology 5357**

**Chemistry & Physics of Biological Molecules**

**Exam #1 Answer Key**

Protein Structure, Folding & Design  
X-Ray Crystallography  
NMR Spectroscopy

**October 20, 2009**

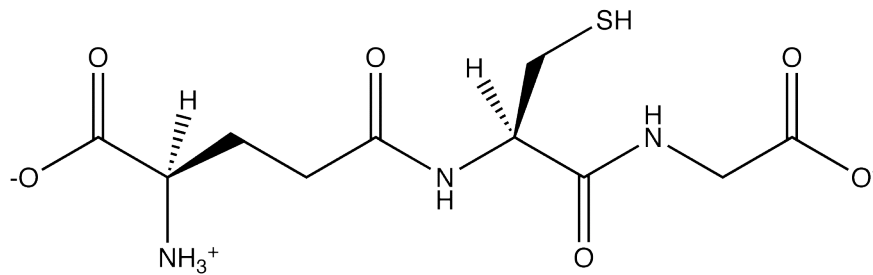
**1. (A)** Structure (b) is right-handed, as it matches the right-handed twist common to nearly all  $\beta$ -sheets. Thus (b) is the preferred connection for two antiparallel strands of sheet. Structure (a) is left-handed and makes a “longer” connection that fights the twist of the sheet.

**(B)** In three-helix bundles there is no right-handed twist between  $\beta$ -strands to bias toward a right-handed overall structure, so the preference for an overall right-handed motif is much diminished. In fact, given the preferred packing angle of “parallel” helices (approx. +20 degrees), we might even have expected left-handed connections to be favored.

**2.** We know that  $\Delta G = -RT \ln(K) = -2.3 RT \log(K)$ , where log is the base-10 logarithm. So, a perturbation of 0.4 pK<sub>a</sub> units upon mutation corresponds to an energy change of  $(-2.3)(0.59)(0.4) = -0.54$  kcal/mol. Note the protonation of the His and removal of the negatively charged residue both involve a change of a full charge unit. Remember Coulomb’s Law of the interaction of two charges is  $E = 332 q_1 q_2 / DR$ , where E is the energy, the q’s are charges in electron units, R is the distance between the charges in Ang, D is the “effective” dielectric constant, and 332 is a conversion factor such that the energy is in kcal/mol. Substituting the above values gives  $-0.54 = (332)(-1)(+1) / (15)D$ , which yields  $D = 41$ .

**3.** Each water molecule in ice forms four hydrogen bonds, two as an acceptor and two as a donor. Since there are two molecules involved in each hydrogen bond, the total count of hydrogen bonds in a sample of ice will be twice the number of molecules. So an initial estimate of the energy associated with each hydrogen bond would be  $12.2 / 2 = 6.1$  kcal/mol. This number might be an overestimate, since dispersive packing effects also contribute to the cohesive energy of ice. If the dispersion energy is about 1 kcal/mol per molecule, then a rough estimate of the enthalpic strength of a hydrogen bond might be about 5 kcal/mol, which is very close to the interaction energy obtained by quantum mechanical calculations.

4. (A) The correct structure is as shown below:



(B) The reduced form of glutathione is a single tripeptide containing a sulfhydryl group as shown in (A). When oxidized, two glutathione monomers connect via a disulfide bond formed between their respective sulfur atoms with the loss of the two sulfhydryl hydrogens.

(C) Glutathione is commonly added to protein refolding buffers as a source of free sulfhydryl and disulfide groups. The presence of these groups can facilitate disulfide exchange within the folding protein, and increase the rate of formation of the correct disulfide pattern in proteins containing native disulfide bonds. The same function is provided *in vivo* by the protein disulfide isomerases such as thioredoxin.

5. (A) The reported values can be obtained via analysis of an equilibrium temperature unfolding experiment. Some spectroscopic or other property is measured as a function of temperature, typically resulting in a sigmoid-shaped plot. After fitting baselines to the low and high temperature regions, the fractions folded and unfolded are computed for the transition region. These fractions are converted to equilibrium constants and then on to free energies at each temperature in the transition region. A plot of  $\Delta G$  vs  $T$  is then curve fit (it would be linear if  $\Delta C_p$  were zero, which is usually not the case). The  $\Delta S$  value will then be the slope of this plot at each  $T$ , and  $\Delta H$  can be obtained via  $\Delta G = \Delta H - T\Delta S$ .

(B) In pure water,  $\Delta G = +7.2 - (298)(23/1000) = +0.35$  kJ/mol. Then using  $\Delta G = -RT \ln(K)$  yields an equilibrium constant for the folded hairpin of 0.87, which converts to 46.5% hairpin. Corresponding

calculations for 50% methanol give  $\Delta G = -4.53$  KJ/mol,  $K = 6.27$ , and an 86.3% hairpin content. Remember that  $1 \text{ cal} = 4.184 \text{ J}$ , and be careful of any unit conversions.

(C) Upon changing the solvent from pure water to 50% methanol, the values exhibit a reduced “hydrophobic effect”. The values in 50% methanol are more like those expected in the “gas phase”, with the  $\Delta H$  being strongly favorable due to hydrogen bonds in the folded form, and the  $\Delta S$  unfavorable due to greater rigidity in the folded vs. unfolded state. In pure water, the  $\Delta C_p$  is much larger, also indicative of a significant hydrophobic effect (*ie*, large temperature dependence of  $\Delta H$  and  $\Delta S$  is a hallmark of the effect).

6. (A) For the F45W mutant with the affinity tag (red dots), the folding arm of the chevron plot crosses the y-axis with a  $\ln(k_{\text{fold}})$  of about +5.7. Extrapolation of the unfolding arm to give its rate in pure water (*ie*, the y-intercept) gives  $\ln(k_{\text{unfold}})$  of -5. Then the equilibrium constant is calculated as  $\ln(K) = \ln(k_{\text{fold}}) - \ln(k_{\text{unfold}}) = 5.7 - (-5) = +11.7$ , or a value of  $K = 4.4 \times 10^4$ . Substitution into  $\Delta G = -RT \ln(K)$  gives a free energy -6.3 kcal/mol.

(B) Since the mutant without the affinity tag is less soluble, it is possible the nonlinearity of its chevron plot at low denaturant concentration is due to aggregation or insolubility (which would lead to a deviation from ideal 2-state behavior). This hypothesis could be tested by redetermining the chevron plot at a series of lower protein concentrations.

7. (A) The space group P1(-) has a center of inversion, which is the common name for a “1-fold screw axis”. Any crystal with such a center of inversion is “centrosymmetric”. Note that both a center of inversion ( $x,y,z \rightarrow -x,-y,-z$ ) and a mirror plane ( $x,y,z \rightarrow x,y,-z$ ) result in inversion of chiral centers. Thus any crystal that has a center of inversion must contain achiral molecules or a racemic mixture of enantiomers. Pure L-plectasin, which is chiral, cannot crystallize in P1(-).

**(B)** For a centrosymmetric space group, and atom at  $(x,y,z)$  requires the presence of a corresponding atom at  $(-x,-y,-z)$ . Plugging both of these positions into the given atomic structure factor equation results in the imaginary portion of the structure factor cancelling to leave only a real portion. Recall the vector diagram for structure factors, where the length of the vector is related to intensity and the rotational angle is the phase in the imaginary plane. Then the only structure factors without an imaginary component will lie along the positive or negative x-axis, *ie*, a phase angle of either 0 or 180 degrees.

**8. (A)** The multiple heavy metal derivatives are needed to “solve the phase problem” and determine an approximate phase value for each reflection. In the limit of exact data, two derivatives are needed to determine phases via isomorphous replacement, but more are often required in practice.  $R$ -factor values are a measure of the agreement between the final, refined structural model and the diffraction data, typically computed as  $R_{\text{cryst}} = (|F_{\text{obs}}| - |F_{\text{calc}}|) / |F_{\text{obs}}|$ .  $R_{\text{free}}$  is a cross-validated  $R$ -factor computed for a subset of the data omitted from the rest of the refinement process.

**(B)** Consider the  $(0,1,1)$  Miller planes as shown in the diagram below. To compute the distance between the  $(0,1,1)$  planes, we first note that  $\tan \theta = 299.67 / 573.77 = 0.522$ , which gives  $\theta = 27.6$  degrees. Using a different right triangle, it is also true that  $\sin \theta = d / 573.77 = 0.463$ , and solving for  $d$  yields 265.6 Ang. Since there are a hundred  $(0,100,100)$  planes between each pair of  $(0,1,1)$  planes, the  $d$  value for the  $(0,100,100)$  planes will be 2.66 Ang, which is the “resolution” of the  $(0,100,100)$  reflection. Bragg’s Law is used to compute the scattering angle for the  $(0,100,100)$  reflection. We know  $\lambda = 2d \sin \theta = 1.0 = (2) (2.65) \sin \theta$ . Solving for the angle gives  $\theta = \arcsin (0.188) = 10.85$  degrees. But the Bragg angle is the angle between the incident (or diffracted) x-rays and the Miller planes. The angle between the incident and the diffracted x-rays is given by  $2\theta$ , or 21.7 degrees.

