250.689 Physical Chemistry of Biological Macromolecules

Assignment #5 – due in class on Monday November 7, 2016

1. The data in the Excel file called TDenat has data for the thermal unfolding of a protein. The data set describes fluorescence vs T. By whatever means possible determine the T_m for this thermal unfolding reaction. T_m is the temperature where half of the protein molecules are folded and half are unfolded. Whatever mathematical manipulations you make must be done with Mathematica. Don't turn this into a more difficult problem than it has to be. It's a matter of finding the inflection point. I won't give you the numerical answer because you can even see what the answer is by eye!
   (a) Describe your procedure with words. If you used an equation, or more than one equation, provide those equations.
   (b) Show a plot of the data indicating Tm, and include in this figure whatever curve or lines if you used them to answer the question.
   (c) Provide the Mathematica Notebook you used to answer this question.

2. The data in the Excel filed called ChemDenatData1 describe the dependence of the intrinsic Trp fluorescence of a highly stable form of staphylococcal nuclease on the concentration of GdnHCl (M) at 15 ^{o}C. Use these data and a two-state model to calculate the thermodynamic stability of nuclease (i.e. ΔG^o in water for the N to U transition). The correct answer is 12.11 kcal/mol so if you do not arrive at this value on your first try, try again. Provide the ΔG^o and the m values you obtained, give the correct units, and provide a sense of the precision with which these parameters were resolved. Provide the following:
   (a) Graph of raw data and the fitted curve.
   (b) Graph of ΔG^o vs [GdnHCl] showing the linear fit extrapolated to [GdnHCl] = 0
   (c) Mathematica Notebook you used to answer this question.

3. What would you do to determine if these data are described correctly by a two state model? Provide a complete and thoughtful answer.

4. What experiments would you perform to establish that the ΔG^o value you measured is meaningful (i.e. how would you show that it is accurate and that it behaves as a function of state?)

5. Normalize the fluorescence data in Problem #2 (i.e. make the highest value = 1 and the lowest value = 0), and fit again. Did the m value or the value of ΔG^o change? Provide a graph showing the normalized data and the fit.

6. How many points can be removed from the native state baseline before the standard deviation in ΔG^o begins to deteriorate? Start from the ends and work towards the C_m. Deal with the N baseline first, then work with the U baseline. Explain and justify your conclusion with ΔG^o values and graphs.

7. How many points can be removed from the transition region before the standard deviation in ΔG^o begins to deteriorate? Start from the C_m and remove points, two at a time, one at higher and one at lower denaturant concentration. Continue to move

towards the baselines in this fashion. Explain and justify your conclusion with ΔG^o values and graphs

8. Introduce random error into the data set in Problem #2 and find out how much error can be tolerated before the error in ΔG^o reaches the 1 kcal/mol level. For the perturbed data set with this level of error provide:
   (a) Graph of raw data and the fitted curve.
   (b) Graph of ΔG^o vs [GdnHCl] showing the linear fit extrapolated to [GdnHCl] = 0
   (c) Mathematica Notebook you used to answer this question.

9. The data in the Excel file called ChemDenatData2 describe the change in intrinsic fluorescence of two proteins, A and B, as a function of denaturant concentration. Which protein is more stable, A, or B, and by how many kcal/mol? Provide the following:
   (a) Graph of raw data and the fitted curve.
   (b) Graph of ΔG^o vs [GdnHCl] showing the linear fit extrapolated to [GdnHCl] = 0
   (c) Mathematica Notebook you used to solve this problem.