Graduate Topics in Biophysical Chemistry – CH 8990 03
Assignment 7

Due Monday, April 28

1. Use the ITC worksheet that was emailed to you to design an experiment in the following situations. The goal of this question is to select parameters that will give you a sigmoidal curve – if your curve only looks linear, you will not be able to extract parameters from it. Your answer should include (a) which component is in the 125 µL syringe and which is in the 1.5 mL sample cell, (b) the initial concentrations of each, (c) the size of each injection, and (d) a plot of your simulation assuming a reasonable value for $\Delta\bar{H}^0$. You may assume that all binding sites are identical and independent. (5 points each)

   a. An exothermic binding reaction, where 2 titrant molecules bind 1 protein molecule. You suspect the K_D is around 30 µM, and neither the titrant nor the protein is limited in solubility.

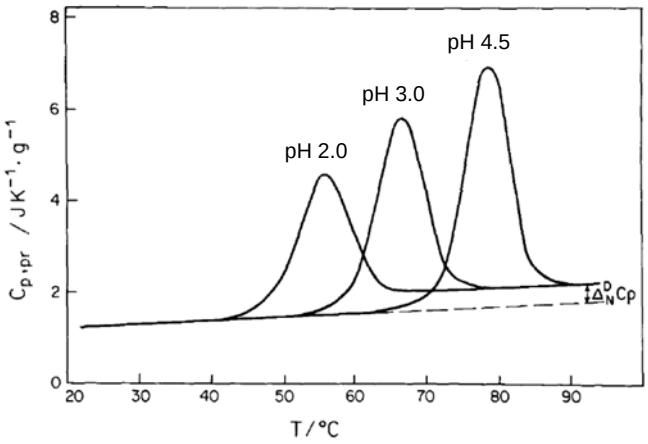
   b. An endothermic binding reaction of 1:4 DNA to ligand. The ligand concentration is limited to 200 µM, and but there are no limitations to the DNA concentration. You expect the K_D to be close to 1 µM.

2. On the website you will find a set of transformed ITC data. The syringe and sample concentrations are 5 mM and 150 µM, respectively, and each injection is 5 µL into a 1.5 mL sample cell. Estimate N, K_D and $\Delta\bar{H}^0$ for this reaction. To solve this problem, you should paste the data into Excel and manually optimize the agreement between the simulated and experimental data. Submit a plot of your fit. If the experiment was performed at 25 °C, what are $\Delta\bar{G}^0$, $\Delta\bar{S}^0$, and $\Delta\bar{H}^0$ for binding? (10 points)

3. van Holde, question 2.10. (5 points)

4. Using DSC, you measure the calorimetric enthalpy of unfolding of a small folding ($\Delta\bar{H}^0_{cal}$) to be 95 ± 8 kcal mol^{-1}. The Van't Hoff enthalpy ($\Delta\bar{H}^0_{VH}$), calculated assuming a two state model, is 73 ± 4 kcal mol^{-1}. What is $\Delta\bar{H}^0_{VH}$, and how could you determine it experimentally? Explain why $\Delta\bar{H}^0_{VH}$ may differ from $\Delta\bar{H}^0_{cal}$. (10 points)

5. Below are a set of DSC curves for a solution of Lysozyme at several pH values:



a. Explain how the curves above relate to the enthalpy of unfolding for each of these curves. (5 points)

b. At pH 4.5, you determine $\Delta\overline{H}^0$ to be 140 kcal mol^{-1}. At pH 2.0, you measure a value for $\Delta\overline{H}^0$ of 122 kcal mol^{-1}. Estimate ΔC_p of unfolding for lysozyme. You may assume that the difference in pH makes no contribution to the thermodynamics of unfolding. *Hint:* Take a look at page 11 of Doug Barrick's DSC notes. (5 points)

7. Give two reasons why hydrogen atoms are difficult to detect with X-ray diffraction. (5 points)