Methods in Biophysical Chemistry – CH 8613
Assignment 7

Due Wednesday, November 30

1. Use the ITC Excel worksheet to design an experiment for the following situations. Recall that, in order to obtain precise fitting parameters, your curve must be reasonably sigmoidal in shape. If the integrated heat looks like a straight line, you will not be able to extract parameters from it. Thus, you should adjust the parameters in the ITC worksheet so that the integrated heads have a fairly flat initial baseline, a reasonably-defined transition at N, and several points beyond where the transition is complete.

   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 90 µM, and neither the titrant nor the protein is limited in solubility.

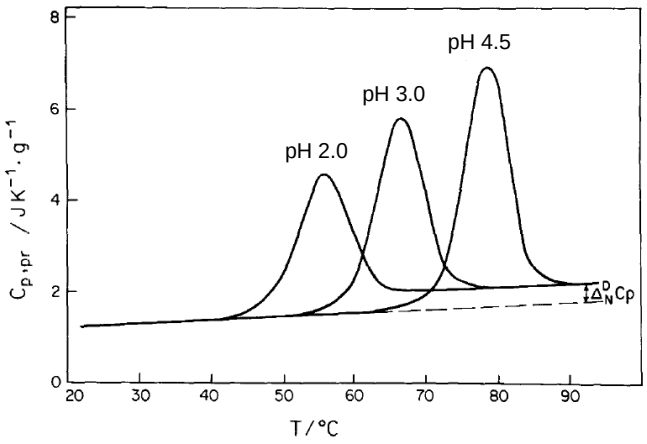
   b. An endothermic binding reaction of 1:4 DNA to ligand. The ligand concentration is limited to 50 µM, and but there are no limitations to the DNA concentration. You expect the K_D to be close to 1 µM, and $\Delta\bar{H}^0$ is expected to be around 20 kJ mol^{-1}.

2. On the website you will find a set of transformed ITC data. The syringe and sample concentrations are 5 mM and 75 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 (by eye) the agreement between the simulated and experimental data. Submit a plot of your fit points and data points (on the same graph). *Hint*: Add another curve to the "Simulation" graph in Excel containing the new experimental data. Then, change the original points so that a smooth line is drawn from the simulated data. Finally, you can adjust the values in column B so that the simulated curve agrees with the "experimental" data. 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. (10 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. Dr. Lewis noted that accurate measurement of $\Delta\bar{C}_p$ can be difficult using DSC. If the baselines are not sufficiently good to determine the heat capacity change, an alternative approach is to perturb the stability using pH and measure enthalpies at several different melting temperatures. At pH 4.5, you determine $\Delta\bar{H}^0$ to be 140 kcal mol^{-1}. At pH 2.0, you measure a value for $\Delta\bar{H}^0$ 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. This is not strictly true, but it works to a first approximation. *Hint:* Take a look at page 11 of Doug Barrick's DSC notes. (5 points)

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