Assignment 4 – Due Friday, 3/09/12, noon.

Thermodynamic Analysis of RNAseA Denaturation by UV-Vis Difference Absorption Spectroscopy (and Differential Scanning Calorimetry).

The accompanying excel file (Origin_Assign_4_Data.xlsx) contains two sets of data. The first set of data represents the change in molar absorptivity at 280 nm (Δe_{280}) versus temperature of ribonuclease A (RNAseA). The second data set are the results of a DSC experiment in which the heat capacity (C_P) was measured as a function of temperature.

***1.** Use the Data for RNase to estimate:*

- ***a.*** *T_M, the transition temperature (where K = [D]/[N] = 1),*
- ***b.*** *ΔH and ΔS of denaturation (at the transition temperature), and*
- ***c.*** *ΔC_P of denaturation.*
- ***d.*** *Provide a write-up that outlines the procedure you used to estimate these parameters. Include (d1) your rationale and procedure for calculating baselines, (d2) the method for determining K as a function of temperature, (d3) a plot of ΔG as a function of temperature, and (d4) the procedure for determining ΔH and ΔS of denaturation (at the transition temperature).*
- ***e.*** *Calculate the temperature of maximum stability.*
- ***f.*** *Generate a protein stability plot (ΔG versus T) based on the fit parameters generated in part **d** and use the plot to predict the T_M of cold denaturation.*

(Assume two-state behavior for RNaseA)

***Extra Credit.** Carry out an analysis of DSC data for RNAse to determine, T_M, ΔH_M, ΔS_M, ΔC_P, the temperature of maximum stability, and the T_M of cold denaturation (T'_G) in that manner that you did for problem 1. In addition to the vant Hoff enthalpy change, calculate ΔH_{cal}, the calorimetric change in enthalpy.*

Conditions of the DSC Experiment
Scan Rate = 1 deg per minute
Cell Volume = 1.411 mL
[RNAse] = 63 μM