** This examination is open book, <u>but is to be worked on **independently**</u>. You may not discuss or otherwise communicate *any* aspect of the exam with *anyone* other than C. Martin. This includes any discussions with anyone after you are done with the exam, but before the exam's due date and time. This is ***very important***.

Due in class, 9:30am, Thursday, May 1

Show your work for full credit. Be concise, but complete.
Avoid long rambling answers which indicate that you don't really understand the question.

1. (30 points) Consider a protein with the following thermodynamic parameters:

   $\Delta H_{298} = -57$ kcal mol^{-1} $\Delta S_{298} = -187$ cal mol^{-1} K $^{-1}$ $\Delta Cp = -2.7$ kcal mol^{-1}

   You introduce a small mutation that weakens the enthalpic stability of the protein by only 1.0!kcal mol^{-1} (less than 2%) at 298K, but has no effect on ΔS_{298} or on ΔCp). Calculate the effect of this mutation on the stability of the protein, ***specifically***, what are the changes in ΔG at 25°C and on the midpoint of the temperature unfolding curve (T_m)?

   You may want to "solve" the T_m part graphically (or not)… Note that T_m is the temperature at which you have equal parts folded and unfolded in solution.

ΔG_{298} (native) =	kcal/mol	T_m (native) =	°C
ΔG_{298} (mutant) =	kcal/mol	T_m (mutant) =	°C

2. (40 points) In the following progression of simulated DSC traces of protein thermal denaturation, parameters (from $C_p^D, C_p^N, \Delta H^o, \Delta S^o$) were changed from one spectrum to the next. Discuss what you know qualitatively about each of the parameters below. In particular, compare parameters from the various traces – how is one parameter different in one DSC trace from that same parameter in the preceding trace? Bigger, smaller, zero, positive, negative, etc?

C_p^D

C_p^N

ΔH^o

ΔS^o

C_p^D

C_p^N

ΔH^o

ΔS^o

C_p^D

C_p^N

ΔH^o

ΔS^o

C_p^D

C_p^N

ΔH^o
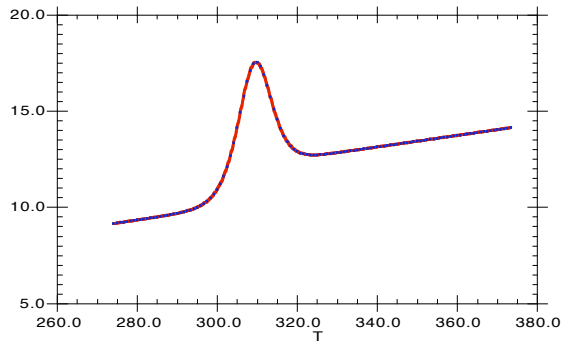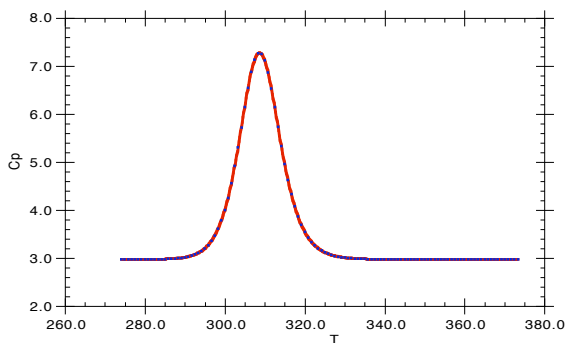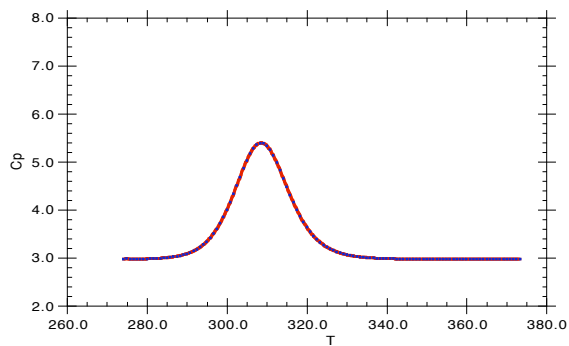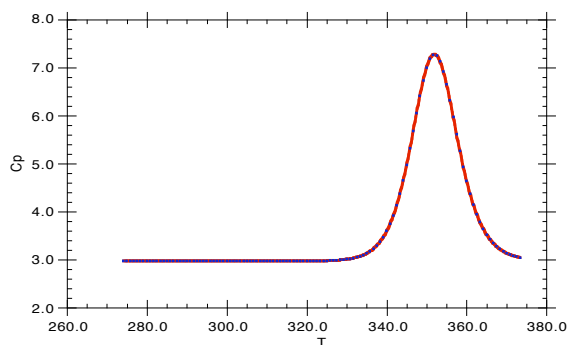
ΔS^o

3. (30 points) The following equation shows the temperature dependency of ΔG for a reaction, assuming zero change in heat capacity.

$$\Delta G(T) = \Delta H - T\Delta S$$

We derived in class the following:

$$\Delta G_{T_2} = \Delta H_{T_2} - T\Delta S_{T_2}$$

$$\Delta G_{T_2} = \left[\Delta H_{T_1} + \Delta C_p(T_2 - T_1)\right] - T_2\left(\Delta S_{T_1} + \Delta C_p \ln\frac{T_2}{T_1}\right)$$

$$\Delta G_{T_2} = \Delta H_{T_1} - T_2\Delta S_{T_1} + \Delta C_p(T_2 - T_1) - T_2\Delta C_p \ln\frac{T_2}{T_1}$$

a) (10 points) Another way of thinking about the temperature dependence of ΔG is to calculate ΔΔG = ΔG$_{T2}$ - ΔG$_{T1}$. Use the above to derive ΔΔG in terms of ΔG$_{T1}$, ΔCp and ΔS$_{T1}$, T1 and T2.

b) (10 points) The current version of the online lecture notes reflect thinking of the past decade that melting of duplex DNA has a "near-zero" change in heat capacity and therefore ΔH and ΔS can be considered to be independent of temperature. More recently, Bloomfield et al, presented arguments to the contrary. Specifically, for a particular oligonucleotide at its melting temperature (72°C), the following parameters were determined:

$$\Delta C_p = 65.3 \frac{cal}{mol\ K} \qquad \Delta S = 24.9 \frac{cal}{mol\ K}$$

Use the equation you derived in (b) to argue that this value of ΔCp is "not negligible." How far off is it?

c) (10 points) Use the equation from part (b) to calculate the value for ΔH at 72°C.