

\*\* This examination is open book, but is to be worked on *independently*. 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:

$$\Delta H_{298} = -57 \text{ kcal mol}^{-1}$$

$$\Delta S_{298} = -187 \text{ cal mol}^{-1} \text{ K}^{-1}$$

$$\Delta C_p = -2.7 \text{ kcal mol}^{-1}$$

You introduce a small mutation that weakens the enthalpic stability of the protein by only

1.0 kcal mol<sup>-1</sup> (less than 2%) at 298K, but has no effect on  $\Delta S_{298}$  or on  $\Delta C_p$ ). Calculate the effect of this mutation on the stability of the protein, *specifically*, what are the changes in  $\Delta G$  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.

$\Delta G_{298}$ (native) =	-1.274 kcal/mol	$T_m$ (native) =	33.7 °C
$\Delta G_{298}$ (mutant) =	-0.274 kcal/mol	$T_m$ (mutant) =	29.4 °C

Note that in determining  $T_m$ , many of you tried the approach that at the melting temperature  $\Delta H = T\Delta S$ . This is correct. However, you cannot simply plug in the values for  $\Delta H$  and  $\Delta S$  from above, as those numbers are only valid at 298 K. This is a key concept. You could either solve this graphically, plotting the full temperature-dependent  $\Delta H$  and  $T\Delta S$  (or  $\Delta G$ ), as many of you did, or you might have tried an iterative approach: first calculate it assuming that  $\Delta H$  and  $\Delta S$  are constant, then reevaluate  $\Delta H$  and  $\Delta S$  at the new temperature and recalculate  $T_m$ . In this way, you would converge on the real value (actually no one tried it that way).

$$\Delta G_T = \Delta H_{T_h} - T\Delta S_{T_s} + \Delta C_p \left[ T - T_h - T \ln \frac{T}{T_s} \right]$$

$$\Delta G_{298}^{\text{native}} = (-57000) - 298(-187) + (-2700) \left[ 298 - 298 - 298 \ln \left( \frac{298}{298} \right) \right]$$

$$\Delta G_{298}^{\text{native}} = (-57000) - 298(-187) + 0$$

$$\Delta G_{298}^{\text{native}} = -57000 + 55726 = -1.274 \text{ kcal / mol}$$

$$\Delta G_{298}^{\text{mutant}} = -56000 + 55726 = -0.274 \text{ kcal / mol}$$

Noting that at the  $T_m$ ,  $\Delta G=0$

$$\Delta G_{T_m} = 0 = \Delta H_{T_m} - T_m \Delta S_{T_m} + \Delta C_p \left[ T_m - T_o - T_m \ln \frac{T_m}{T_o} \right]$$

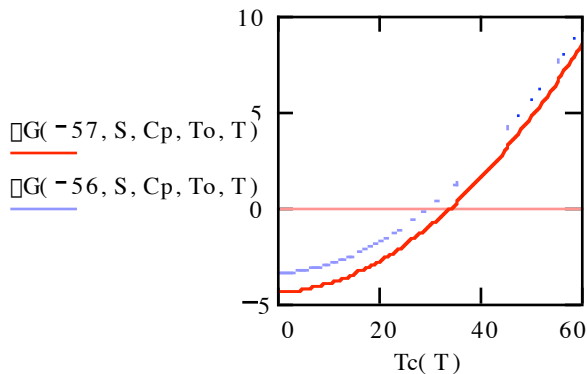
There are ways in MathCAD to solve this numerically, but let's do it graphically:

$$\Delta H(\Delta H_{To}, \Delta C_p, T_o, T) := \Delta H_{To} + \Delta C_p \cdot (T - T_o) \quad \Delta S(\Delta S_{To}, \Delta C_p, T_o, T) := \Delta S_{To} + \Delta C_p \cdot \ln\left(\frac{T}{T_o}\right)$$

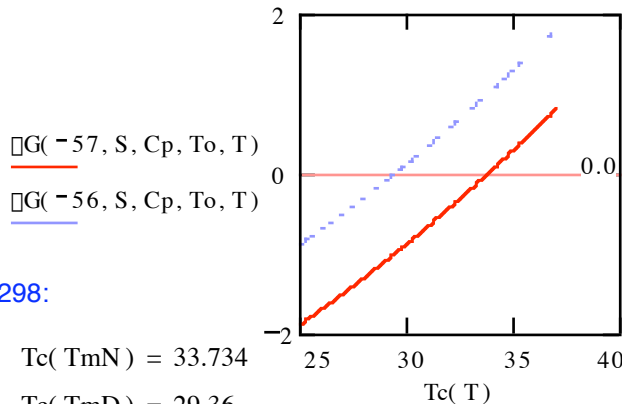
$$\Delta G(\Delta H_{To}, \Delta S_{To}, \Delta C_p, T_o, T) := \Delta H(\Delta H_{To}, \Delta C_p, T_o, T) - T \cdot \Delta S(\Delta S_{To}, \Delta C_p, T_o, T)$$

Use graphical analysis to estimate solution:

$$T := 273, 273.5.. 333 \quad T_c(\text{TK}) := \text{TK} - 273 \quad H := -57.0 \quad S := -0.185 \quad C_p := -2.0 \quad T_o := 298.0$$



$$T := 298, 298.1.. 310$$



Use Matcad's root solving function, with a starting guess of 298:

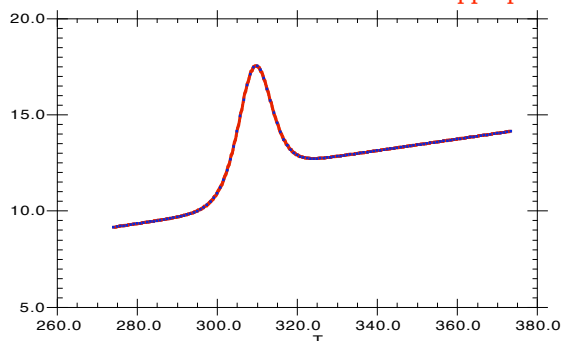
$$T := 298$$

$$T_{mN} := \text{root}(\Delta G(-57, S, C_p, T_o, T), T) \quad T_{mN} = 306.734 \quad T_c(T_{mN}) = 33.734$$

$$T_{mD} := \text{root}(\Delta G(-56, S, C_p, T_o, T), T) \quad T_{mD} = 302.36 \quad T_c(T_{mD}) = 29.36$$

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?

For your info, I've included below each plot, the parameters used to simulate each curve (has to do with T-dependence). With enough MathCAD simulation, you should be able to closely replicate them. All units below: kcal/mole where approp.



$C_{pD}=3.0, C_{pN}=1.0, \Delta b=0.03, \Delta b=0.03, \Delta H=57, \Delta S=0.185, T_0=303$

$C_p^D$

dependent on T (sloping baseline)

$C_p^N$

dependent on T,  $\Delta C_p \neq 0$ . Indeed,  $\Delta C_p = 2.0$  kcal/mol

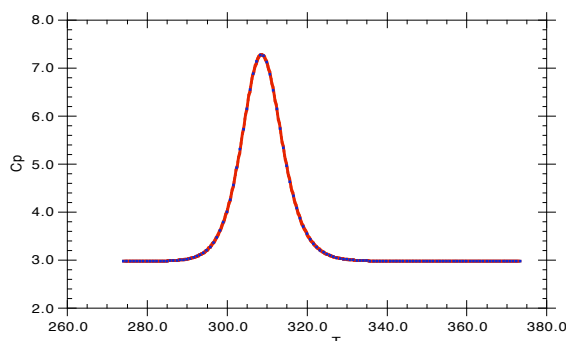
$\Delta H^o$

positive

temperature dependent (because  $\Delta C_p \neq 0$ )

$\Delta S^o$

positive



$C_{pD}=3.0, C_{pN}=3.0, \Delta b=0.0, \Delta b=0.0, \Delta H=57, \Delta S=0.185, T_0=303$

$C_p^D$

Now independent of T (flat baseline)

$C_p^N$

Now independent of T,  $\Delta C_p = 0$

$\Delta H^o$

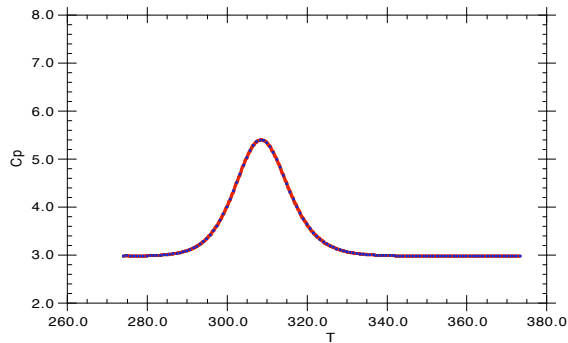
Same magnitude as above, though it's hard to tell without simulating. Positive.

Temperature independent ( $\Delta C_p = 0$ )

$\Delta S^o$

Still positive

Temperature independent



$C_{pD}=3.0$ ,  $C_{pN}=3.0$ ,  $\Delta C_p=0.0$ ,  $\Delta C_p=0.0$ ,  $\Delta H=42.75$ ,  
 $\Delta S=0.138$ ,  $T_0=303$

$$C_p^D$$

Still independent of T

$$C_p^N$$

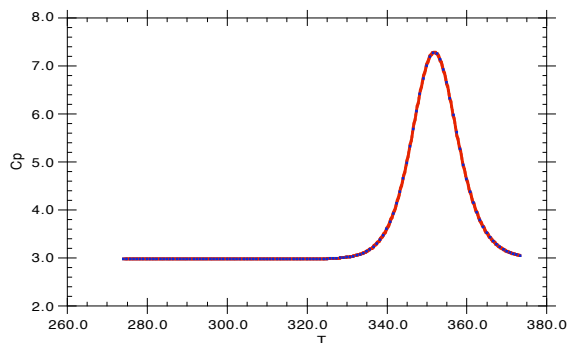
Still independent of T.  $\Delta C_p = 0$ .

$$\Delta H^o$$

Now clearly smaller than before (area under curve is smaller). Still positive.

$$\Delta S^o$$

$T_m$  hasn't changed, so since  $\Delta H$  smaller,  $\Delta S$  must also be smaller.



$C_{pD}=3.0$ ,  $C_{pN}=3.0$ ,  $\Delta C_p=0.0$ ,  $\Delta C_p=0.0$ ,  $\Delta H=65$ ,  
 $\Delta S=0.185$ ,  $T_0=303$

$$C_p^D$$

$$C_p^N$$

$$\Delta H^o$$

Clearly larger than immediately above.

$$\Delta S^o$$

Since  $T_m$  is shifted to higher T,  $\Delta S$  has not increased in the same proportion that  $\Delta H$  has increased.

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

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

We derived in class the following:

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

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

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

- a) Another way of thinking about the temperature dependence of  $\Delta G$  is to calculate  $\Delta\Delta G = \Delta G_{T_2} - \Delta G_{T_1}$ . Use the above to derive  $\Delta\Delta G$  in terms of  $\Delta G_{T_1}$ ,  $\Delta C_p$  and  $\Delta S_{T_1}$ ,  $T_1$  and  $T_2$ .

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

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

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

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

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

$$\Delta\Delta G = \Delta G_{T_2} - \Delta G_{T_1} = \left( \Delta C_p - \Delta S_{T_1} \right) (T_2 - T_1) - T_2\Delta C_p \ln \frac{T_2}{T_1}$$

- b) 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  $\Delta H$  and  $\Delta S$  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:

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

Use the equation you derived in (a) to argue that this value of  $\Delta C_p$  is “not negligible.” How far off is it?

In a typical use of this approach, we would measure the thermodynamics near the melting temperature for the duplex, 72°C. We might want to then take those parameters and calculate back to the thermodynamics of DNA melting at room temperature ( $T=25^\circ\text{C}$ ). Let's calculate  $\Delta\Delta G$ . ( $T_1=345\text{K}$ ,  $T_2=298\text{K}$ ) we have.

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

$$\Delta G = (65.3 - 24.9) \frac{\text{cal}}{\text{mol K}} (T_2 - T_1) + T_2 \left[ 65.3 \frac{\text{cal}}{\text{mol K}} \right] \ln \frac{T_2}{T_1}$$

$$\Delta G = \left[ 40.4 \frac{\text{cal}}{\text{mol K}} \right] (T_2 - T_1) + T_2 \left[ 65.3 \frac{\text{cal}}{\text{mol K}} \right] \ln \frac{T_2}{T_1}$$

$$\Delta G = \left[ 40.4 \frac{\text{cal}}{\text{mol K}} \right] (298\text{K} - 345\text{K}) + (298\text{K}) \left[ 65.3 \frac{\text{cal}}{\text{mol K}} \right] \ln \frac{298\text{K}}{345\text{K}}$$

$$\Delta G = \left[ 40.4 \frac{\text{cal}}{\text{mol K}} \right] (-47\text{K}) + (298\text{K}) \left[ 65.3 \frac{\text{cal}}{\text{mol K}} \right]$$

$$\Delta G = \left[ -1899 \frac{\text{cal}}{\text{mol}} \right] + \left[ 2850 \frac{\text{cal}}{\text{mol}} \right] = 951 \frac{\text{cal}}{\text{mol}} = 0.95 \frac{\text{kcal}}{\text{mol}}$$

Assuming zero  $\Delta C_p$  and using the value of  $\Delta H$  from part (c) below,

$$\Delta G = \Delta H - T \Delta S = 8.59 \frac{\text{kcal}}{\text{mol}} - (298\text{K}) \left[ 0.0249 \frac{\text{kcal}}{\text{mol K}} \right] = 8.59 \frac{\text{kcal}}{\text{mol}} - 7.42 \frac{\text{kcal}}{\text{mol}} = 1.17 \frac{\text{kcal}}{\text{mol}}$$

By most measures, 0.95 is significantly different from 1.17 (23% different!).

c) Calculate the value for  $\Delta H$  at 72°C.

This is the  $T_m$ , so  $\Delta G=0$  at 72°C

$$\Delta G = \Delta H - T \Delta S = 0$$

$$\Delta H = T \Delta S = (345\text{K}) \left[ 24.9 \frac{\text{cal}}{\text{mol K}} \right] = (345\text{K}) \left[ 24.9 \frac{\text{cal}}{\text{mol K}} \right] = 8.59 \frac{\text{kcal}}{\text{mol}}$$