Solutions to Problem Set 4

**Problem 1**
Melting curve and $\Delta G$ unfolding vs. temp for wild-type protein (top) and mutants (bottom)

b) signal for WT: folded 1.7, unfolded 2.8  mutant: folded 1.75, unfolded 2.6
d) melting temperatures 307 K for wild-type and 309 K for mutant
e) used the $\Delta G$ vs. temp plot (see above)
f) $\Delta \Delta G$ at 25 deg. C (298K) = 0.69 kcal/mol for wild-type and 0.66kcal/mol for mutant protein. (These stability values are extremely low for a protein, I guess I did not pick very good numbers for this example.
g) fraction of protein folded at 20 deg C equals $1-((A_{fold} - A_{obs})/(A_{fold} - A_{unfold})) = 84\%$
h) 0.1% of folded protein corresponds to a $K_{eq} = (1-0.001)/0.001$. We convert this to $\Delta G$ by $\Delta G = -RT \ln K_{eq}$, With $\Delta H = 23.8$ kcal/mol and $\Delta S = 0.0777$ kcal/(mol*K) and $-RT \ln K_{eq} = \Delta H - T \Delta S$ we can solve for $T$ by dividing both sides by $T$ and rearranging to get: $\Delta H / (\Delta S - R \ln K) = 373$ deg K.

This is of course assuming that $\Delta H$ and $\Delta S$ are independent of temperature etc. etc. etc.

**Problem 2**

a) For the mutant Tm=330 deg K; $\Delta H_{unfold}=31.56$ kcal/mol; $\Delta S_{unfold}=96$ cal/(mol*K) and $\Delta G$ at 20 C° = 3.52 kcal/mol. $\Delta \Delta H = -0.44$ kcal/mol; $\Delta \Delta S = 6$ cal/(mol*K) and $\Delta \Delta G = -2.11$ kcal/mol.

b) The fraction of unfolded protein at 20 deg would be very low so we would have to find an incredibly sensitive assay for the unfolded protein. However, you can make a
reasonable estimate of the fraction of unfolded protein by calculating the fraction of unfolded protein from the $\Delta G_{\text{unfold}}$ at 20°C = 3.52 kcal/mol. $K_{eq} = e^{(-\Delta G/RT)} = f_{\text{unfold}}/(1-f_{\text{unfold}})$ rearranging we get $f_{\text{unfold}} = e^{(-\Delta G/RT)/1+ e^{(-\Delta G/RT)}} = 0.002 = 0.2$

c) I would not have expected to see an effect of the mutant. The residue is in an aqueous environment in both the unfolded and folded form of the protein. So there should be no change upon folding. However, we observe a jump in the entropy of unfolding. In other words entropy is driving the unfolding of the protein. One possibility is that in the mutant the unfolded protein is actually not completely unfolded, but that the phe sidechain forms hydrophobic interactions with some other side chain in “unfolded” state of the protein. This hydrophobic effect would show up in the form of an increased entropy of unfolding. Obviously this is just one of many hypotheses that could explain the observations. The main point is that the mutation did not yield the expected results.

Problem 3

a) Probably the best residues to replace the two methionines to form a salt bridge are lysine and aspartic acid. Arginine is out of the question because of its bulky head group and glutamic acid would be one carbon to long to work optimally with lysine.

b) Lets assume that the charges on either residue involved in the salt-bridge are localized on only one atom and lets further assume that the radii of those atoms is 1.5 Å. Finally lets assume that the interior of the protein has a dielectric constant of 6 (if you assumed for example that it was 4 or 8 it would also be correct). Hopefully you remember that the energy of burying a salt bridge was the same as the energy of burying a single charge. So we get our transfer energy by $(333*q^2/(2r))(1/6 - 1/78) = 17.07$ kcal/mol. This energetic contribution should be completely enthalpic in nature. (We could in principle take into account the 0.8*2 kcal/mol we loose by removing two hydrophobic methylene groups from the core of the protein, but we will neglect this relatively subtle effect here.)

c) The wild-type protein has $\Delta H_{\text{unfold}} = 52$ kcal/mol and $\Delta S_{\text{unfold}} = 160$ cal/(mol*K) so $Tm = \Delta H_{\text{unfold}}/\Delta S_{\text{unfold}} = 325$ K = 52 C. For the mutant protein the $\Delta H_{\text{unfold}}=52-17=35$ kcal/mol and an unchanged $\Delta S_{\text{unfold}} = 160$ cal/(mol*K) so Tm of the mutant is $Tm = 35/0.16 = 218$ K = -54 C. In other words the protein solution freezes before the mutant protein would fold.

d) The energetic calculations that we did above indicate that there is no chance that the mutant could fold by forming and burying a salt-bridge. This is not surprising since most proteins have free energies of unfolding of less than 17 kcal/mol under physiological conditions. So our mutant design is not well-suited for testing the energetic cost of burying a salt bridge. So if the mutant does indeed fold, we can be all but certain that the mutation has introduced substantial structural rearrangements that have placed the
residues involved in the salt bridge into a less hydrophobic environment than the protein interior.
So if the protein does indeed fold, it would make a lot of sense to determine its structure, before declaring that burying salt bridges is not as energetically unfavorable as previously thought.

**Problem 4**

a) wildtype: $\Delta H_{\text{unfold}} = 23$ kcal/mol, $\Delta S_{\text{unfold}} = 0.07$ kcal/(mol*K)

mutant: $\Delta H_{\text{unfold}} = 16$ kcal/mol, $\Delta S_{\text{unfold}} = 0.048$ kcal/(mol*K)

b) we do the calculation the same way we did in question 2b

$f_{\text{unfold}} = e^{(-\Delta G/RT)}/1+ e^{(-\Delta G/RT)}$ → at 37 deg C  wildtype: 11% unfolded ;  mutant: 14% unfolded.

The melting temperatures are: wild-type:328 K;  mutant: 333 K

Our calculations reconfirm our observations. Less of the wild-type protein is unfolded at 37 deg C, but the melting temperature of the mutant is higher.

c) Our results perfectly explain what initially appeared to be a paradox. The mutant has a much lower entropy of unfolding than the wild-type protein. This gives the mutant a much more gradual melting curve. So even at relatively low temperature we already have a significant fraction of molecules unfolded (14% at 37 deg. C) for the same reason the temperature has to rise a lot until we reach the 50% point of unfolding (i.e. the melting temperature). In this sense a general statement that one protein is more stable than another can be misleading. To be precise, one would have to specify the precise conditions, under which one wants to compare the stability of two proteins.