

Answers to Problem Set #4:

1. A. The plot is figure 1 from an excellent review on the stability of thermophilic proteins (*Protein Science*, **15**, 1569-1578 '06). Plugging $T = T_m$ into the given equation yields $\Delta G(T) = 0$. But $\Delta G(T) = -RT \ln(K)$, so when $\Delta G = 0$, $K = 1$. To show the T_S and T_H relations, just start from $\Delta G(T) = -RT \ln(K) = \Delta H - T\Delta S$. The maximum protein stability will be at the T where ΔG is at a maximum. To find this temperature, note the derivative of ΔG with respect to T is just ΔS . So ΔG will be maximized when ΔS is 0, which is the definition of T_S . Similarly, after some simple algebra and calculus, we find the derivative of K with respect to T is $(\Delta H/RT) \exp(-\Delta H/RT + \Delta S/R)$, which will be equal to 0 when ΔH is 0. Thus, K is maximal when ΔH is 0, or at T_H .

B. Just from the shape of the plot, we can see that the curve bends downward, so linear extrapolation of ΔG based on the slope of the curve in the near-linear region around T_M will overestimate the protein stability at room temperature. The linear extrapolation would be exactly correct if ΔC_p were equal to 0.

2. A. The graph shown is figure 2 from the same review article cited in problem 1. The “diamond” plot raises the entire stability curve and corresponds to method (a) in the problem description. The “circle” plot broadens the curve so it intersects the x-axis at a higher temperature and is method (b). Finally, the “square” plot shifts the curve to the right and corresponds to method (c).

B. The temperature dependence of the free energy of protein folding is similar to that for the hydrophobic effect. At very low temperature, the exposure of hydrophobic surface is less costly due to a decreased entropic penalty for formation of clathrate structure adjacent to hydrophobic surfaces. This same mechanism could destabilize folded proteins at low temperature.

3. A. The YibK structure contains some β - α - β motifs and is generally considered to be a member of the alpha/beta fold family.

B. The structure contains a formal “knot” in the region near the C-terminus, *ie*, if you grab the two ends of the amino acid chain and pull, the structure forms a knot. This is very rare among known protein structures.

5. A. Just estimating from the chevron plot, it looks like the WT has a folding rate of about 120 (*ie*, $\ln(k) = 4.8$, so $k = 121.5$). Similarly, we estimate the folding rate for L108A to be 100. Extrapolating the unfolding arms to where they intersect the y-axis at 0M urea gives an unfolding rate of about 0.01 for WT and 0.13 for L108A. Then, the equilibrium constant is $K(\text{WT}) = 12000$, corresponding to a ΔG of -5.5 kcal/mol. For L108A, we have $K = 770$ and $\Delta G = -3.9$ kcal/mol. So the WT protein is more stable than L108A by about 1.6 kcal/mol. In making these linear extrapolations, we are implicitly assuming a 2-state folding reaction.

B. The L108A mutation has little effect on the left branch of the chevron plot, the folding rate. But the mutation has a big effect on the unfolding rate. This suggests that the effect of the mutation is manifested relatively late in the folding process.

6. Using $\Delta G = \Delta H - T \Delta S = 0$, plugging in the values from the table and solving for T yields T_m values of 48.9 C, 55.2 C and 44.3 C for the WT, mutant 1 and mutant 2, respectively. Then we can use the relation $\Delta\Delta G = T_m \Delta\Delta S$ and an average value for ΔS of 315 cal/mol/K to conclude that mutant 1 is stabilized relative to WT by 2.0 kcal/mol, while mutant 2 is destabilized by just under 1.5 kcal/mol.

7. A. The data suggests that thermophilic organisms increase protein stability by increasing the stability of the folded state (as opposed to decreasing stability of the unfolded form).

B. Simply extrapolate each of the folding and unfolding branches to find their intercept with the y-axis, exactly as in problem 5. Then take the ratio of the rates to find the equilibrium constant for each protein. Finally, use $\Delta G = -RT \ln(K)$ to compute the corresponding $\Delta G_{U \rightarrow N}$ values. As before, we are making a 2-state folding assumption.

8. A. L-Ala has a methyl group as its side chain. D-Ala would have the mirror image Ramachandran map. The allowed regions on the map for Aib, which has both the L- and D-Ala side chain methyls, would be approximately the intersection of the L-Ala and D-Ala maps. Thus, the only allowed regions for Aib will be small regions centered near the standard L-amino acid α -helix and its mirror image D-residue α -helix.

B. From the answer to **A**, the presence of Aib residues should enforce a helix, either an L- or D- α -helix. If L-amino acids are also present in the sequence, then we would expect a standard L- α -helix. Indeed, Aib residues tend to induce α -helix structure in short peptides.