Answers to Problem Set #3:

(1) The data in the problem is taken from *Biochemistry*, *32*, 183–190 (1993). The stability of the folded state relative to the unfolded state is given by multiplying the [GdnHCl] at the transition midpoint by the slope, m, to give a ΔG extrapolated to 0M denaturant. The ΔG values are wild type (5.66 kcal/mole), Met 73 (4.66), Tyr 73 (4.61), Phe 73 (4.51) and Trp 73 (4.23). Theoretical analysis suggests that the slope of the free energy vs. [denaturant] plot is proportional to ΔA, where $\Delta A = A_u - A_f$, the difference in solvent–exposed surface of the unfolded and folded states. See *Biopolymers*, *17*, 1305–1322 (1978) for the analysis. The authors of the *Biochemistry* paper interpret their results in terms of increased relative stability of the unfolded state for the hydrophobic mutants due to interactions in a compact "denatured" state.

(2) This problem is based on a paper from Dahlquist's group at the University of Oregon (the first author is Jirong Lu, who was later a postdoc in Kathleen Hall's group at Washington U). The reference is *Biochemistry*, *31*, 7765–7772 (1992). From the data given, formation of the mixed disulfide with cystamine lowers the stability of the protein by about 1.1 kcal/mole. Using a thermodynamic cycle like that shown in figure 4 of the paper, the authors conclude that 0.9 kcal of the energy difference is reflected in destabilization of the folded form, and 0.2 kcal in stabilization of the unfolded form.

(3) The data illustrates inactivation and disulfide interchange of ribonuclease S–protein by the enzyme disulfide isomerase. The S–peptide prevents inactivation if present initially, by forming the stable ribonuclease S complex. The S–protein has the four original disulfide bonds and retains a folded conformation with some similarities to native ribonuclease A. It unfolds, however, if interchange of the four disulfides is

allowed, as in the presence of disulfide isomerase. The S–peptide can reverse the disulfide rearrangement of S–protein, presumably by pulling the disulfide equilibrium toward the form of the S–protein with the folded conformation and the correct disulfides. The plot is adapted from a classic paper by Kato and Anfinsen, *J. Biol. Chem.*, *244*, 1004–1007 (1969).

(4) The *de novo* design of an α/β barrel protein has been reported! See Protein Engineering, 3, 259–266 (1990) for a discussion of the design process and initial characterization of synthetic "octarellin".

(5) The basic idea behind "chevron plots" is described in the short review by Matthews and Hurle, *BioEssays*, *6*, 254–257 (1987). This is highly recommended, and fairly simple, reading. The figure in the question is taken directly from a recent "chevron" analysis of T4 lysozyme mutants by Schellman, et. al. in *Biochemistry*, *31*, 1464–1476 (1992). See in particular figure 7 and its corresponding discussion for the authors' "answer" to the question.