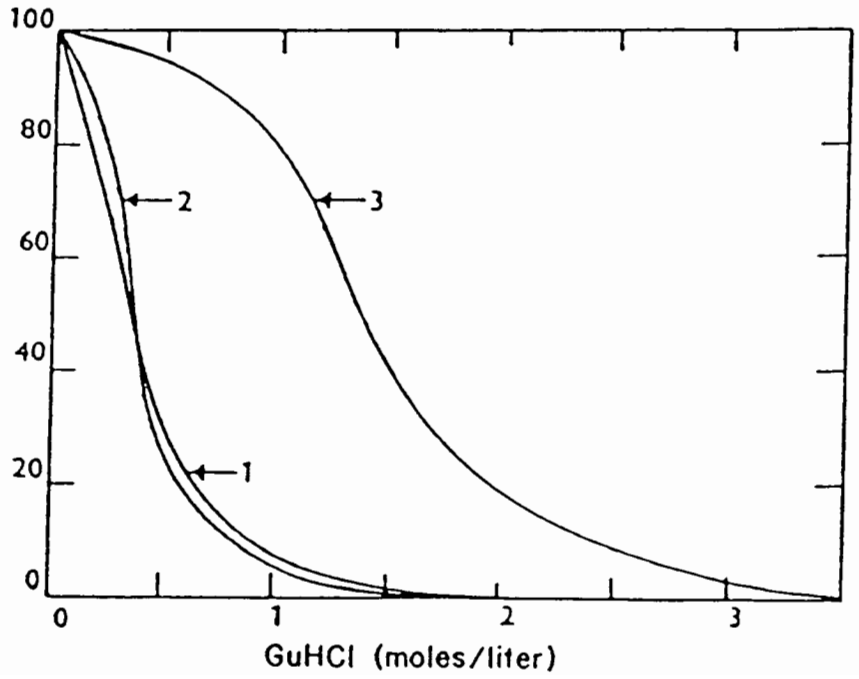
Problem Set #2:

(1) The protein penicillinase from *Staphylococcus aureus* can be unfolded by high concentrations of standard denaturants. The figure below shows the unfolding of penicillinase as a function of [GuHCl] when monitored by three different methods. Curve 1 is a plot of UV absorbance at 278 nm, near the λ_{max} for aromatic residues. Curve 2 monitors the increase in viscosity of the protein solution as a function of increasing [GuHCl]. Curve 3 is the molar ellipticity at 220 nm as measured by circular dichroism (CD) spectroscopy. All three properties have been normalized to a scale of 0 to 100 for presentation on the same plot.

(a) What does this data imply about the validity, for penicillinase, of the "two–state" assumption often made in the analysis of such data?

(b) Do these unfolding curves provide support for any of the generic models for the protein folding process?



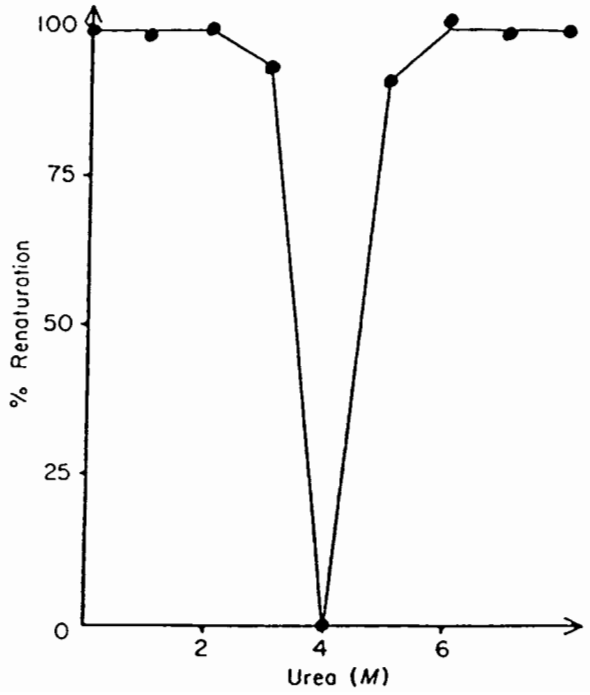
(2) Analysis of the thermal unfolding of a protein is generally similar to the analysis of denaturant unfolding data, but can yield some additional information. For the following problem, remember that $\Delta G = \Delta H - T\Delta S$, $\Delta G = -RT \ln(K)$, $0^{\circ}C = 273.2K$ and $R = 1.987$ cal/mole/degree.

(a) Assuming that ΔH and ΔS are independent of temperature (ie, that ΔC_p is zero), what is the rate of change of ΔG with respect to temperature (ie, what is the derivative $d(\Delta G)/dT$)?

(b) A thermal unfolding curve for ribonuclease T1 gave a melting temperature, T_m, of $48.3^{\circ}C$. Upon further analysis of the raw data, a plot of ΔG vs. T was produced and shown to have a slope of –300.3 cal/mole/degree at T_m. What are the values of ΔG_m, ΔH_m and ΔS_m; the free energy, enthalpy and entropy change for the unfolding process at the melting temperature?

(c) Thermal unfolding data is often analyzed by making a van't Hoff plot (ie, plotting ln(K) vs. 1/T). If the van't Hoff plot were to yield a straight line, what values could we ascribe to the slope and intercept? The van't Hoff plots of protein unfolding transitions are found to be nonlinear. Why?

(3) Nine samples of correctly folded β–galactosidase were fully denatured by exposure to 8M urea. The different samples were then diluted to a common protein concentration of 100 μg/ml and to different urea concentrations ranging from 0M to 8M in 1M increments. After incubating for 1 hour, each sample was returned to renaturing conditions in the complete absence of denaturant. The amount of refolded protein was determined. The plot below shows the percentage of protein that refolded as a function of the urea concentration during the incubation period. The apparent irreversibility of the refolding process after exposure to 4M urea is both time and concentration dependent. If the incubation time is made very short, then refolding is observed even for the sample incubated in 4M urea. If the whole experiment is repeated with a protein concentration during incubation of 500 μg/ml, then the failure to refold extends to both higher and lower urea concentration. A standard urea unfolding curve suggests that β–galactosidase is essentially completely unfolded in 4M urea. Suggest an explanation for these data.



(4) The partioning of the tryptophan side–chain analogs 3–methylindole (3–MI) and N–methylindole (NMI) between cyclohexane and water was recently reported. Shown below are the measured values of the volume–fraction partition coefficient K_v, which is equal to the ratio of the molar concentrations of the solute in cyclohexane and in water.

Temp	K_v for 3–MI	K_v for NMI
288	18.6	301
298	19.1	290
308	19.7	274

Use these values to compute the free energy, enthalpy and entropy of transfer of each solute from water to cyclohexane. What conclusions can be drawn from the thermodynamic values. How do the values compare with other data as presented in the class lectures? Will the results be altered by the presence of water in the cyclohexane phase? How might you correct for this effect?

(5) The Generalized Born/Surface Area (GB/SA) solvation model first proposed by Still, *et. al.* (J. Amer. Chem. Soc., *112*, 6127–6129, '90) is one of the most successful continuum solvation models. Briefly describe the components of the GB/SA model. What are the assumptions made in using this model? For what sorts of molecular systems might you expect the GB/SA model to be most appropriate? least appropriate?