Problem Set #3:

(1) Recently, a group at Colorado State has replaced lysine–73 of Cytochrome C with a series of hydrophobic amino acids. Position 73 is evolutionally–conserved and has a relatively high degree of solvent exposure in the native state. The crystal structure shows the ammonium group of lysine–73 to be at least 12 Å from any other charged protein group. The side chain of position 73 has no obvious interaction with any other protein side chain. The guanidinium unfolding of the wild–type protein and position 73 mutants was followed by circular dichroism at 220 nm. Given below is the guanidinium concentration ($[GdnHCl]_{1/2}$) and slope of the unfolding curve at the midpoint of the transition (m) for each protein. What is the standard interpretation of the "m" value derived from this sort of equilibrium unfolding experiment? Rationalize this data in terms of the relative structure and energetics of the folded and unfolded states of Cytochrome C?

Mutant	$[GdnHCl]_{1/2}$ (M)	m (kcal/mol/M)
Wild–type	1.16	4.87
Met 73	1.05	4.43
Tyr 73	1.00	4.62
Phe 73	1.02	4.44
Trp 73	1.02	4.16

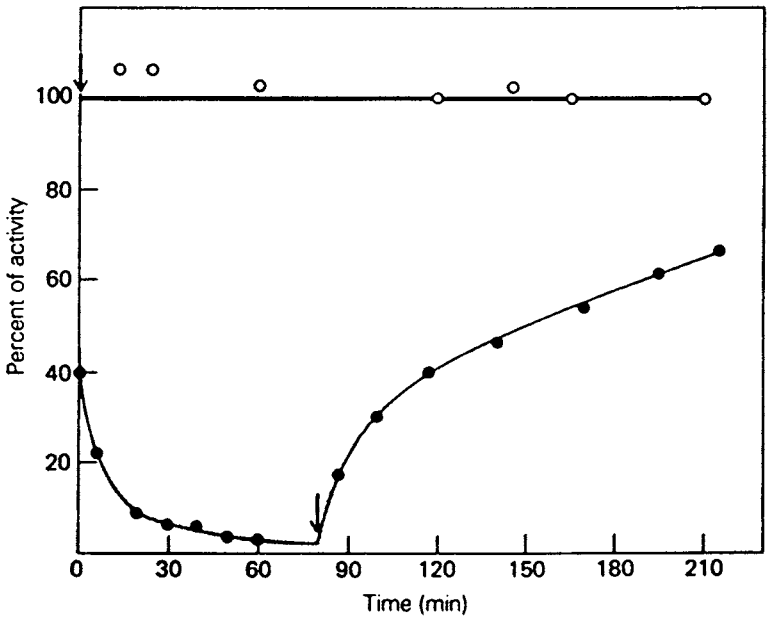
(2) Proteins containing a single cysteine residue (P–SH) are subject to the following exchange reaction with cystamine ($^+NH_3CH_2CH_2S–SCH_2CH_2NH_3^+$, or RS–SR) :

$$P–SH + RS–SR \underset{}{\overset{K}{\rightleftharpoons}} P–S–SR + RSH$$

The equilibrium constant for this reaction with cystamine has been determined at pH 5.14 and 56°C for both the folded and unfolded forms of the T4 lysozyme A146C mutant, and for

N–acetylcysteinamide (NACA) as a model system for a noninteracting Cys residue. For folded A146C the researchers report K = 0.59, unfolded A146C gives K = 3.1, and NACA gives K = 2.2. Use thermodynamic cycle arguments to describe the effect of coupling to cysteamine (RSH) on the stability of both folded and unfolded T4 lysozyme A146C.

(3) Ribonuclease A is a protein of 124 amino acid residues. It contains four disulfide bonds, formed between residues 26–84, 40–95, 58–110 and 65–72. The native protein can be cleaved proteolytically between residues 20 and 21 to give two fragments: S–peptide (residues 1–20) and S–protein (residues 21–124). Upon mixing equimolar amounts of S–peptide and S–protein, they associate to form a noncovalent complex with a structure very similar to native, uncleaved Ribonuclease A. The plot below shows the effect on nuclease activity of treating Ribonuclease A (open circles) and S–protein (closed circles) with the enzyme Protein Disulfide Isomerase. In the case of S–protein, 1.3 equivalents of S–peptide were added to the mixture after 80 minutes (indicated by the arrow). Explain these results.



(4) Describe the kinds of design principles you would employ if asked to design an eight–stranded α/β barrel protein sequence. If you could produce unlimited amounts of the proposed sequence, what methods would you employ to test your design?

(5) Bob Matthews' lab at Penn State has pioneered the use of "chevron curves" in the analysis of protein folding pathways. Typical chevron plots are made by plotting the log of the relaxation time as a function of denaturant. The relaxation time (t), assuming a simple two state process, is just the reciprocal of the sum of the rate constants for unfolding and refolding. This is represented by the following scheme:

$$N \underset{kf}{\overset{ku}{\rightleftharpoons}} U \qquad kr = 1 / \tau = ku + kf$$

Chevron plots have been determined for T4 lysozyme mutants at position 3. Note that this is the same position that Brian Matthews, *et. al.* used to test the effect of placing different residues into a small hydrophobic pocket of the protein.

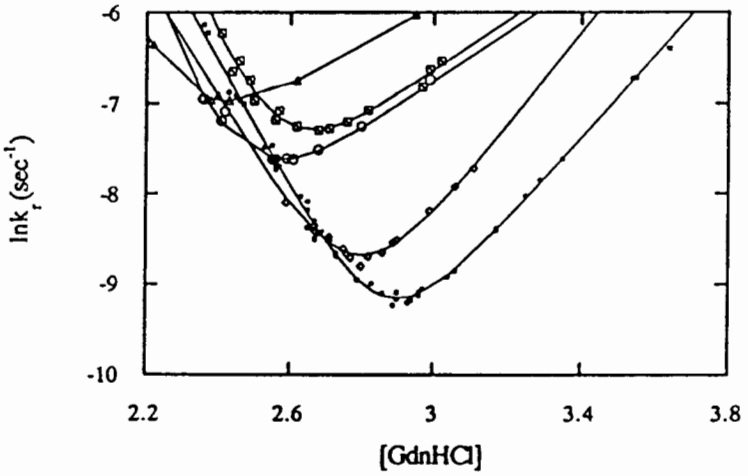


FIGURE 6: The first-order kinetics of mutations at position 3: I3V (◇), I3F (□), I3A (○), I3G (△), and the wild type (■).

What do these plots tell us about the effects of mutations at position 3 on the folding of T4 lysozyme? Does this data make sense in light of Brian Matthews' X–ray structures and stability measurements (see *Nature* 334 406–410 '88) for these same mutants?