(1) Define or uniquely describe the following terms or phrases:

(a) Heirarchical folding (b) Burst phase

(c) Chaperone (d) Energy landscape

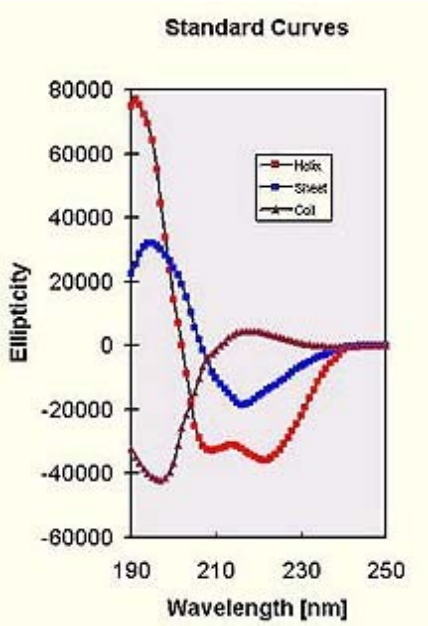
(2) Propose an experiment(s) to demonstrate the role of surface residues in protein folding?

(3) How is pulsed H/D exchange NMR used to follow protein folding?

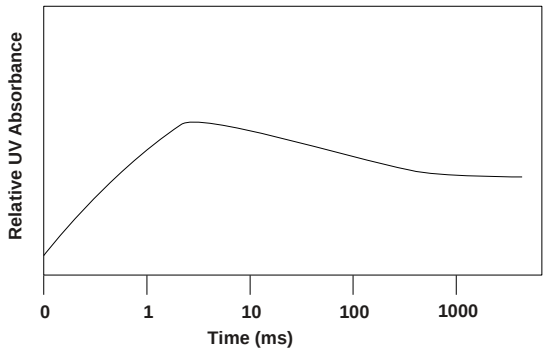
(4) For a number of small, disulfide rich proteins, the rate and efficiency of protein folding is dramatically increased by protein disulfide isomerase (PDI). How does PDI increase the rates and efficiency of folding?

(5) What is the role of the 'trans' ring in GroEL-GroES function?

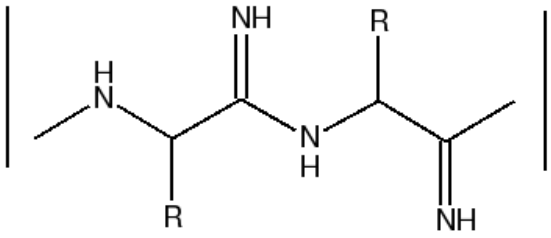
(6) Calculate the helical content of protein sample with a molar ellipticity of -26000 at 222 nm? (use the figure to the right)



(7) You have run a 'stop-flow' experiment in which one syringe contains denaturant and protein and the second syringe contains a solvent system that allow protein folding. You have followed your experiment by following small changes in the UV absorbance as a function of time. Interpret the following 'stop-flow' spectra.



(8) You are supervising a brilliant, yet erratic young scientist who has been working on the development of novel peptide-based inhibitors. As part of their work, they developed isostructural (same shape) amino acid analogues that form non-hydrolyzable 'peptide' bonds (NH replaces O of carbonyl group; see below).



Thrilled with the success of their very short petide-based inhibitors, they propose synthesizing a known polypeptide chain (expensive and challenging but possible) using the novel amino acid analogues. Do you think the proposed synthetic polypeptide analogue will fold under normal physiological conditions? Why or why not.

Answers:

1a – Folding involves the association of local segments into secondary structures, followed by approximate motif, subdomain and domain structure formation. The final stages of protein folding involve heirarchical structure orientations and side chains adopting their folded state conformations.

1b – This is the earliest protein folding event and it involves the rapid collapse (< 0.1 ms) of the polypeptide into a compact globular state or molten globule. The hydrophobic core and most secondary structures have formed but have not adopted their folded state conformations.

1c – One class of folding accessory protein that minimizes misfolding. There are multiple classes of chaperones including chaperonins, Hsp70s, Hsp90s and nucleoplasmins.

1d – Energy landscapes are 3D plots describing protein folding pathways. The xy plane in the landscape represents all possible protein conformations while the z axis represents the free energy the conformation.

2 - Any one of the following experiment approaches would be reasonable:

(i) A series of site-directed mutations of surface residues could be generated and each tested for the ability to fold. This could then be compared to similar mutations in non-surface residues.

(ii) Systematic chemical modification of surface residues would exploit the same concept as in (i). Alternatively, a series of insertions on the surface of proteins could be introduced and tested for their ability to fold in comparison to insertions within the hydrophobic core.

(iii) Known denaturants could be tested to examine which residues that interact with and/or which non-covalent forces they disrupt.

3 – Hydrogen is NMR active (detectable) while deuterium is not. Further, main chain amine exchange rates are significantly lower for (1) residues in the hydrophobic core and (2) residues that are part of secondary structures. (Note: can only be measured for samples adopting stable partially folded state)

A denatured protein sample containing H atoms only is introduced into conditions that stabilizes a folding intermediate (*ie.* molten globule) and contain a deuterated solvent. Under these conditions, the remaining NMR resonances will be those residues that are part of the hydrophobic core, secondary structures or both.

(Note: there are several additional ways in which H/D exchange can be used to follow folding)

4 – PDI preferrentially breaks incorrect disulfides and allows correct disulfide to form. Correct disulfides are typically buried within the hydrophobic core of proteins and are not substrates for PDI. In contrast, incorrect disulfides typically prevent correct folding or are located on the surface of partially folded proteins. In either case, incorrect disulfides are available substrates.

5 – The 'trans' ring of GroEL is essential for the release of ADP, GroES and the refolded protein from the 'cis' ring. Once ATP has been hydrolyzed to ADP in the 'cis' ring, misfolded protein and ATP bind to the 'trans' ring. In addition to the release ('cis' ring) and binding ('trans' ring) events, GroEL undergoes a concerted conformational change with the 'cis' ring adopting the more closed conformation

and the 'trans' ring the more open conformation that facilitates GroES binding.

6 – To estimate the helical content we must estimate the helical content of the random coil (0% helix) and all helix (100% helix) polypeptides on the standard curve. Assuming a molar ellipticity of -12000 (0% helix) and -38000 (100% helix) respectively, the helical content is
(-12000 - -26000) / (-12000 - -38000) = 14000/26000 = 0.54 or 54%

7 – The trace shows three phases.

a) Initially the absorbance rises to a maxima after a few milliseconds. This is consitant with the hydrophobic collapse of the denatured polypeptide into a molten globule.

b) The absorbance then slowly decreases to a minima near 1000 ms. This is consistant with the slow conversion of the molten globule into a natively folded state.

c) Finally, the absorbance reaches a constant value that likely represents the absorbance of the natively folded state.

8 – Given the only change to the polypeptide structure is the swap of all carbonyl oxygens with an amine, we must consider the role of carbonyl oxygen atoms in protein structure. As carbonyl oxygen atoms are an integral component of hydrogen bond interactions in secondary structures we must consider how the presence of an amine will alter this interaction. N is less electronegative than O and the partial negative charge associated with the N atom will be correspondingly lower. At pH's were the N is neutral (ie. As in the diagram), the main chain hydrogen bonds it forms would tend to be weaker and secondary structures would be less stable. Further, the main chain hydrogen bonds would likely be more costly to bury within the hydrophobic core as the amine (replacing the carbonyl oxygen) would be less effective at neutralizing the partial charge associated with the α-amine. Overall, it seems clear that the synthetic polypeptide would be less stable than the naturally occurring polypeptide, though it is unclear if these destabilizing effects are large enough to produce a denatured synthetic polypeptide.

(Note: For hypothetical questions like this one, the bulk of the marks are awarded for the logic of your arguments as opposed to saying folded or unfolded)