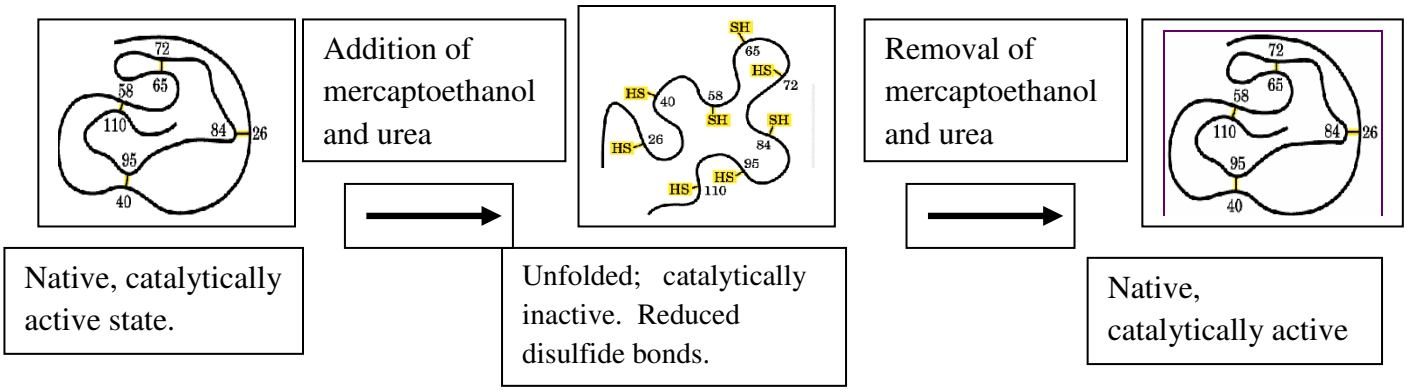
BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI
BIO C331, BIOPHYSICS
FIRST SEMESTER 2010 – 2011
COMPREHENSIVE EXAMINATION (CLOSED BOOK)
Full marks: 100 (Weightage: 30%) DATE: 06.12.10 DURATION: 3 Hrs.

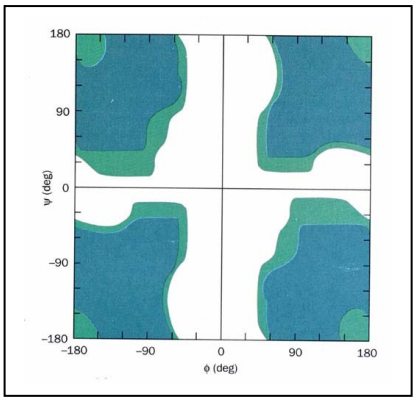
- **Answer to the point**
- **Irrelevant answer may attract penalty**
- **Steps in each calculation carry marks**
- **Answer PART-A and PART-B in separate answer paper**

PART-A

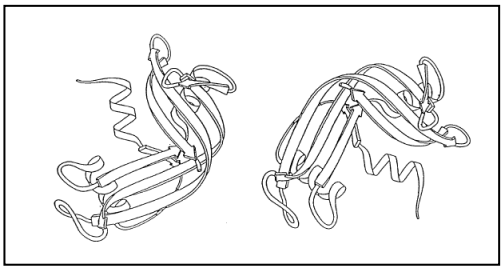
1. a) Attached sheet contains fluorescence and CD study of apoflavodoxin protein which contains three tryptophan and six tyrosine residues. For what purpose the researcher designed these experiments? After examining the spectral data, discuss the structural properties as well as folding kinetics of the given protein. From the given structure of Guanidinium hydro chloride, explain how does GndHCl or Guanidinium hydro chloride, one of the strongest denaturants denature proteins.
b) From the given schematic representation of famous Anfinsen's experiment and your knowledge of protein folding theory explain how a protein folds very quickly into native conformation. **[10+5]**



2. a) Suppose you are asked to develop hydrophobicity scale of naturally occurring amino acid side chains. Describe your strategy and point out the possible drawbacks. Why do some hydrophobicity scales show cysteine (Cys) as most hydrophobic residue? **[3+2+2]**
b) The following Ramachandran plot showed the allowed region of polyglycine (by shaded area). Take any phi (ϕ) and psi (ψ) torsion angle of disallowed region and explain pictorially (considering di-glycine as an example) the reason of disallowed conformation. **[3]**



c) Which of the two structures shown below is the correct structure for Ribonuclease A? List as many structural reasons as you can to justify your choice. **[3]**



d) Some natural proteins are rich in disulfide bonds, and their mechanical properties (tensile strength, viscosity, hardness, etc.) are correlated with the degree of disulfide bonding. What is the molecular basis for the correlation between disulfide-bond content and mechanical properties of the protein? **[2]**

e) Segments of the Fos and Jun proto-oncogene proteins have been shown to form heterodimeric coiled coils of two helices in solution. The "leucine-zipper" portion of the sequences that are thought to directly interact given below

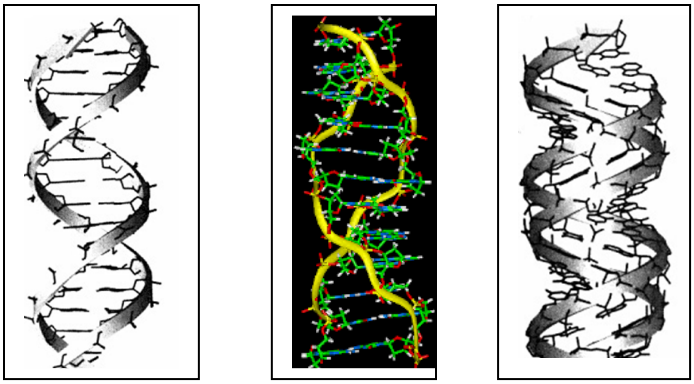
Fos: LQAETDQLEDKKSALQTEIANLLKEKEK

Jun: LEEKVKTLKAQNSELASTANMLREQVAQ

Plot each sequence in the helical wheel diagram and identify the contact region of coiled coil of two helices. **[5]**

3.a) Bacteriophage λ infects *E. coli* by integrating its DNA into the bacterial chromosome. The success of this recombination depends on the topology of the *E. coli* DNA. When the superhelical density (σ) of the *E. coli* DNA is greater than -0.045, the probability of integration is <20%; when σ is less than -0.06, the probability is >70%. Plasmid DNA isolated from an *E. coli* culture is found to have a length of 13,800 bp and an *Lk* of 1,222. Calculate σ for this DNA and predict the likelihood that bacteriophage λ will be able to infect this culture. **[3]**

b) Identify A, B and Z DNA from the given figure. What are the features by which you identify? List down all other features of these three forms of DNA**.** **[4+2]**



c) It is observed that most of the recognition and interaction between biomolecules are through weak forces. Why does nature choose such mode of communication? **[3]**

d) Why C2'-endo and C3'endo pucker are two most stable sugar puckers in deoxyribose sugar? Explain with the help of figure. **[3]**

4 a) What is the basis of molecular mechanical (MM) energy calculation of biomolecules? Why do MM energies have no meaning as absolute quantity? **[2+2]**

b) What are parameters on which following properties of biological membrane depend?

(i) Fluidity **(ii)** Permeability **(iii)** Stability **(iv)** Specificity **[4]**

c) Write short notes on

(i) DNA grooves **(ii)** Class of protein structure **(iii)** UV spectroscopy in protein analysis **[3]**

d) In the process of transcription, RNA chain is synthesized with the help of RNA polymerase in presence of DNA strand. In this process either the polymerase must screw around the DNA or the DNA thread must screw itself through a stationary polymerase. Which of these alternatives will be favored in living cells? Justify your answer with proper reasons. **[4]**

PART-B

1. By taking proper assumptions **[3]** and schematic diagram **[2]** evaluate the partition function for a polypeptide chain forming α-helix **[10]**. Discuss the terms appearing in the expression explicitly.

2. By working out the proper steps and figure **[3]** find out an expression for the **S** (the number of cycles per radiation) as a function of incident angle θ **[5]** in the X-ray diffraction experimental setup**.**

3. Discuss the important outcome (pointwise) **[5]** of the experiment in which a dsDNA is being stretched in a dual beam optical tweezers. Draw proper schematic of the experiment **[3]** and phase diagram **[4].**

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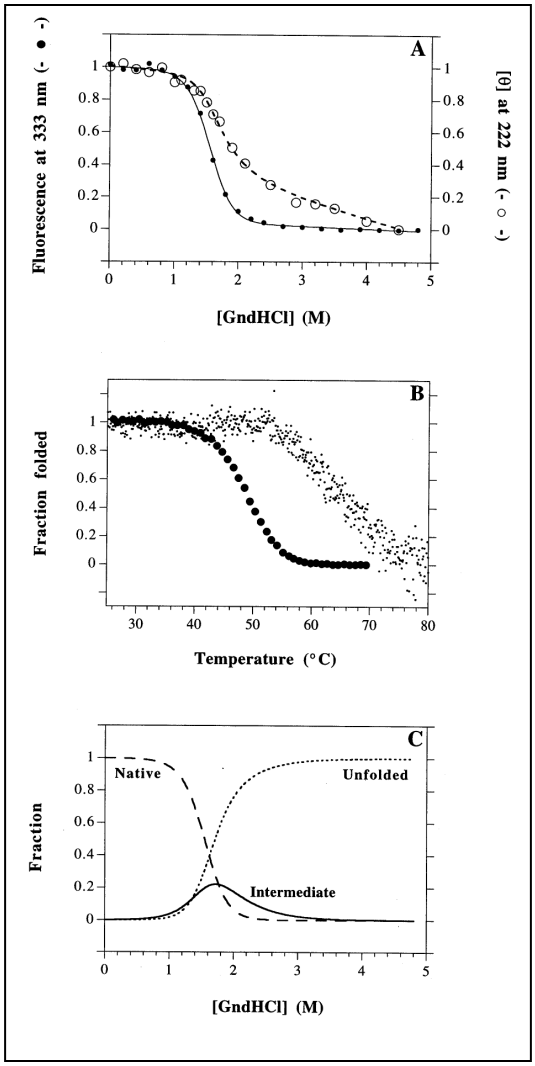
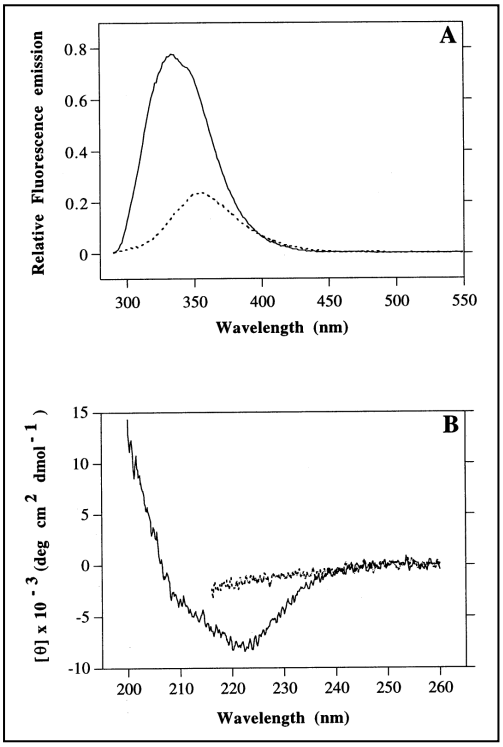
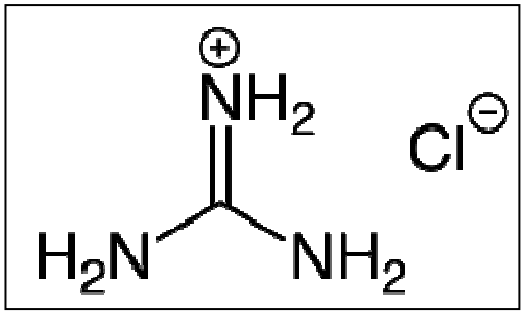


(A) GndHCl-induced unfolding profiles as measured by fluorescence at 333 nm (•) and by ellipticity at 222 nm (o). Results of the fits for the changes in fluorescence and ellipticity calculated for a three-state transition are shown in solid and dashed lines, respectively. (B) Fraction folded C69A apoflavodoxin molecules versus temperature as monitored by the change in fluorescence emission at 350 nm (•) (the fluorescence excitation wavelength was 280 nm) and by the change in CD at 222 nm (small black dot). Both fractions are calculated using the fit parameters of a fit of a two-state model of unfolding to the individual apoflavodoxin thermal unfolding curves. (C) Simulated fractions of native (- - -), intermediate state (—), and unfolded (· · · ·) C69A apoflavodoxin molecules as a function of the concentration GndHCl for a three-state apoflavodoxin unfolding transition. The apoflavodoxin concentration was :4 mM (A) or 2 mM (B). Apoflavodoxin was in 100 mM potassium pyrophosphate, pH 6.0, and the spectra (A) were recorded at 25°C. The heating rate (B) was 0.5°C min_1.



(A) Fluorescence emission spectrum of native C69A apoflavodoxin (—) and of apoflavodoxin in 4 M GndHCl (- - -), respectively. The excitation wavelength was 280 nm.
(B) Far-UV CD spectrum of native C69A apoflavodoxin (—) and of apoflavodoxin in 4.2 M GndHCl (- - -), respectively. The protein concentration was: 4 mM and the spectra were recorded at 25°C in 100 mM potassium pyrophosphate, pH 6.0.



Guanidinium Hydro chloride