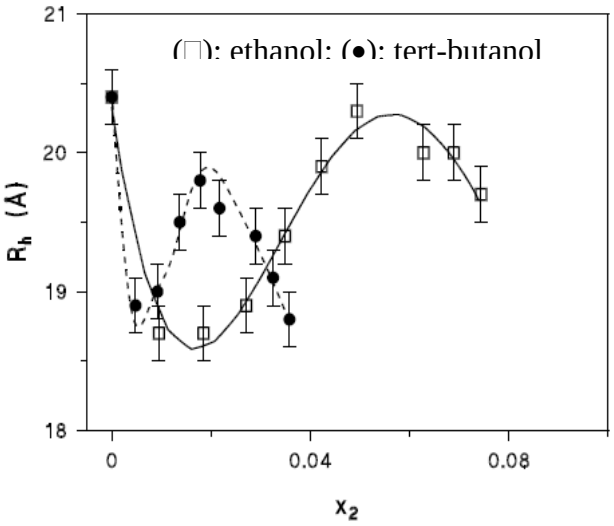
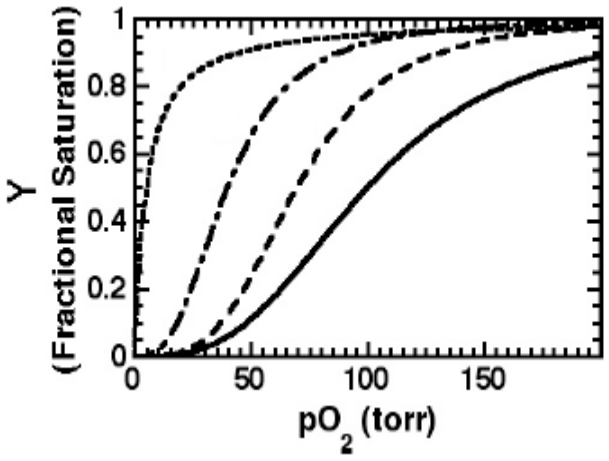
INDIAN INSTITUTE OF TECHNOLOGY, KHARAGPUR

Subject No. CY60004 Time: 3 hrs Subject Name: Biophysical Chemistry
Spring Semester 2007-2008 Final year M.Sc. No.of students: 18
Instructions: Graph paper required for Q1(c) Full Marks: 60

Q1(a) The figure on the left shows the effect of alcohol on the hydrodynamic radius, R_h of lysozyme in water/alcohol mixtures as a function of alcohol mole fraction x_2. Explain how this plot may be obtained from a light scattering experiment. What conclusions can you draw from the nature of the curves obtained in the two cases?
(b) What is a Hill plot? Draw a Hill-plot for an enzyme with negative cooperativity.
The plots on the right show the relationship of fractional saturation (Y) to oxygen concentration (expressed as pO_2, in torr). Label each curve as A, B, C or D based on the following information.
Curve A: normal adult human hemoglobin under conditions in which P_{50} = about 40 torr.
Curve B: normal adult human hemoglobin at a lower pH than curve A.
Curve C: a mutant human hemoglobin which exists only in the R conformation; it cannot undergo a conformational shift to the T state.
Curve D: a mutant human hemoglobin in which the T⟶R conformational equilibrium is strongly shifted toward the T state.





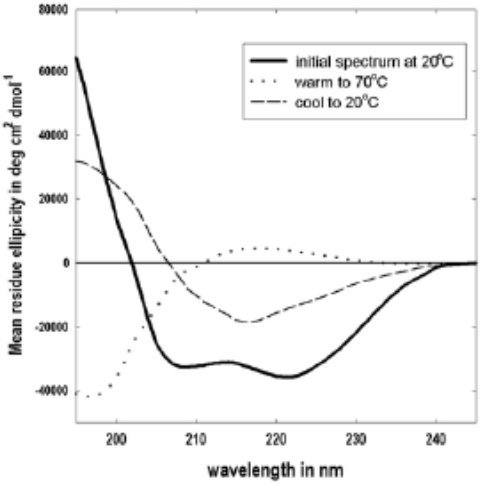
(c) A series of Equilibrium Dialysis Experiments were set up where a fixed concentration and volume of the protein is contained within dialysis tubing and "dialyzed" until an equilibrium is reached against a solution containing varying concentrations of a certain ligand. This allows the "free" ligand to reach an equilibrium between the inside and outside regions of the experiment, while the "bound" ligand is contained entirely inside the dialysis tubing. From the following data, calculate the dissociation constant for the ligand binding to the protein. How many binding sites per native molecule are there? [Protein Concentration in Experiment: 0.918mg/ml; Polypeptide Molecular Weight of Protein: 55,800; Molecular Weight by Gel Filtration: 335,200]

Tube #	***Total Ligand Conc. Inside Tube***	***Total Ligand Conc. Outside Tube***
1	12.80	8.90
2	7.65	4.20
3	5.32	2.48
4	2.38	0.70
5	1.60	0.43
6	1.10	0.26
7	0.75	0.18

[6+6+6=18]

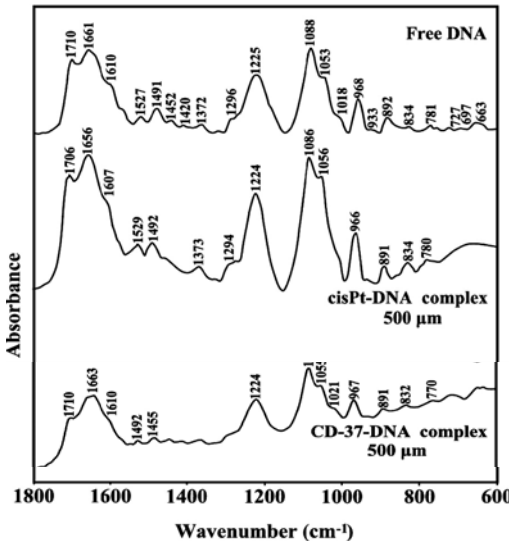
Q2 (a) What is the difference between circularly and linearly polarized light?
(b) What information can you get from the CD spectra of proteins?
(c) The circular dichroism spectra shown are for an experiment where a peptide was initially analyzed at room temperature. The sample was then warmed to 70^0C. On cooling back to room temperature the CD spectrum does not come back to its original value. From the results what can you stay about the changes in the conformational of the peptide on heating and subsequent cooling?

[4+4+4=12]

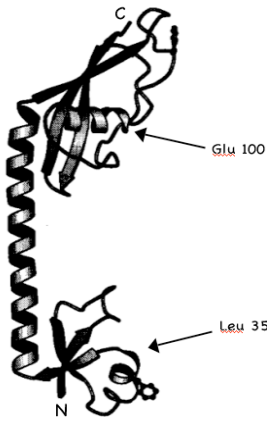


Q3 (a) Which vibrations are important in the study of the FTIR of proteins?
(b) FTIR also has the advantage of resolving the vibrational information originating from the different constituents of the nucleotide into different frequency regions in the FTIR spectrum. What is the information that may be obtained from FTIR spectra of nucleotides?
(c) From the spectra given alongside and the information given below comment on the implications of the spectral changes observed upon drug–DNA complexation for CD-37 and Cis-Platin. [guanine 1710 cm^{-1} ; thymine 1661cm^{-1}; adenine 1610cm^{-1}; cytosine at 1491 cm^{-1}]

[5+5+5=15]



Q4 (a) What methods would you consider using to study the thermal stability of a protein with a single buried tryptophan residue and why?
(b) The structure of a protein determined by x-ray crystallography was found to have a very elongated structure. It is possible to use FRET to determine whether the protein structure in solution is similar to the crystal structure. To accomplish the FRET experiment, amino acids Leu 35 and Glu 100 in the protein that normally contains no cysteines were replaced by Cys residues and fluorophores attached. The distance (r) between the side chain gamma carbon of amino acid Leu 35 and the side chain gamma carbon of Glu 100 in the protein crystal is about 45 Å.



(i) Comment on why this replacement is considered to be convenient for the FRET experiment. How would these mutations be referred to symbolically?
(ii) Fluorophore A (at position 35) has an excitation wavelength of 270 nm, and a fluorescence emission wavelength of 340 nm. Fluorophore B (at position 100) emits at 390 nm when it receives a FRET signal from fluorophore A. R_0 is typically 23 Å for a FRET signal from fluorophore A to B. Assuming that the crystal structure for the protein is an accurate representation of the protein structure in solution, what efficiency of FRET signal would you expect to observe between fluorophores A and B?
(iii) If a FRET efficiency of 0.17 (or 17 percent) is observed between fluorophores A and B, what would this suggest is the distance between the fluorophores? Suggest a possible explanation for the discrepancy between this observed FRET and the expected FRET.

[5+10=15]