ADMISSION NUMBER: **STUDENT NAME:**

FINAL EXAM **SBT 5107 BIOMOLECULAR INTERACTIONS** **DATE: 16TH APRIL 2018**

ATTEMPT ALL QUESTIONS

Qu. 1 (a) What is the "Levinthal Paradox" in the context of protein folding ? Describe how it arises and discuss its implications for the mechanism of protein folding.

[6]

(b) Thermal stability studies have given the following (partial) thermodynamic data for unfolding of a protein in aqueous solution at pH 7.4 at different temperatures:

t /°C	*K*	Δ*G*° /kJ mol^{-1}	Δ*H*° /kJ mol^{-1}	Δ*S*° /J K^{-1} mol^{-1}
45	0.133	5.33	150.0	?
50	?	2.86	175.0	?
55	?	0	200.0	609.8
60	3.22	?	225.0	?

(i) Complete this table by supplying the missing data (?) where possible.
(ii) What fraction of the protein molecules would be unfolded at 50,55 and 60°C, respectively, under these conditions ?
(iii) What does the temperature dependence of the unfolding enthalpy (ΔH°) suggest about the forces responsible for stabilizing the folded protein conformation ?

[10]

(c) The complete genome sequence of a simple nematode worm (*C. elegans*) has just been completed. One of the major tasks now is to identify the function of many of the gene products. Glasgow scientists have identified one protein that might have metal-binding properties. Give three different biophysical techniques that might be used to investigate the binding of metal ions to this protein in solution. In each case describe the theoretical basis of the method and indicate how thermodynamic information may be derived.

[9]

[Gas constant R = 8.314 J K^{-1} mol^{-1} ; zero of the Celsius scale = 273.15 K]

Qu. 2 (a) Describe the molecular basis for some of the anomalous properties of liquid water and explain the significance of this with regard to hydrogen bonding and hydrophobic interactions in biomolecules.

[6]

(b) The dimerization of N-methylacetamide in solution has frequently been used as a model system for inter-peptide hydrogen bonds in proteins. Some data for the dimerization equilibrium constant (K) and enthalpy of dimerization at 25°C in various solvents are given below:

Solvent	K /M^{-1}	ΔG° /kJ mol^{-1}	ΔH° /kJ mol^{-1}	ΔS° /J K^{-1} mol^{-1}
CCl_4	4.7	?	-17.6	?
Dioxane	0.52	?	- 3.3	?
Water	0.005	?	0	?

Supply the missing thermodynamic data in the table (?) and explain what these data might suggest about the role of H-bonding in biomolecular interactions.

[7]

(c) Describe three different experimental techniques that may be used to monitor the unfolding of a protein molecule with change in temperature. In each case explain the physical basis for the method and the nature of the results that might be observed. How could the Gibbs free energy of unfolding be determined from such measurements ?

[7]

(d) What is meant by the heat capacity increment (ΔC_p) for protein unfolding ? How might it be measured and what is it significance to understanding the forces responsible for protein folding stability ?

[5]

Qu. 3 (a) List and explain some of the anomalous properties of water, and describe how they might relate to the forces responsible for stabilising protein and other biomolecular structures.

[7]

(b) Describe experimental techniques using model compounds that might be used to obtain information about the thermodynamics of hydrophobic interactions and hydrogen bonding in the context of protein folding.

[6]

(c) The following experimental data have been obtained for the fluorescence intensity (F) and circular dichroism intensity (CD) of a protein solution at different temperatures.

T /°C	F (arbitrary units)	CD (arbitrary units)
20	65.0	-1310
30	65.0	-1310
40	64.7	-1304
46	58.8	-1186
50	40.0	-810
56	17.8	-366
60	15.5	-320
70	15.0	-310
80	15.0	-310

What is the T_m of this protein ?
What fraction of the protein might be unfolded at 46 °C, and what is the Gibbs free energy of unfolding at this temperature ?

[7]

(d) Explain what molecular properties are being monitored by the two different sets of data in (c). Do the transitions monitored by fluorescence and CD necessarily have to occur at the same temperature ? If not, explain why not.

[5]

[Gas constant R = 8.314 J K^{-1} mol^{-1} ; zero of the Celsius scale = 273.15 K]

Qu. 4 (a) Describe, with appropriate examples and definitions, how the thermodynamics of hydrogen bonding and hydrophobic interactions can be studied experimentally by use of small model compounds.

[8]

(b) Discuss the role of hydrogen bonding and hydrophobic interactions in stabilising the folded conformations of globular proteins. What is the current view regarding the relative importance of the contributions from these two interactions ?

[5]

(c) There is currently considerable concern regarding the environmental levels of plasticisers and their potential effects on male sexual development. Why might these compounds be of concern ?

[3]

(d) A series of organic compounds with increasing levels of methyl group substitution, $X\text{-}(CH_2)_n\text{-}CH_3$, are under investigation as potentially more environmentally-friendly plasticisers. Using group-additivity data, the following values have been obtained for the predicted free energy of transfer of such compounds from cyclohexane to water at 25 °C :-

n	$\Delta G°_{transfer}$(cyclohexane→water) / kJ mol^{-1}
2	- 0.2
4	+ 6.9
8	+ 21.1

Calculate the partition (distribution) coefficient, D, for each of these compounds, defining carefully what you mean by this quantity.

[6]

Discuss how these data might affect your choice of which compound might be the best choice for its use as a plasticiser, and how such molecules might bind to hydrophobic binding sites on transport proteins and hormone receptors.

[3]

[Gas constant R = 8.314 J K^{-1} mol^{-1} ; zero of the Celsius scale = 273.15 K]

Qu. 5 (a) List and describe briefly the different non-covalent interactions thought to be involved in stabilizing protein folding and protein-ligand binding interactions in solution. In each case, discuss the role of solvent water and how it might affect the interaction.

[6]

(b) What are the typical thermodynamic features for thermal unfolding of a globular protein in solution and how are they determined experimentally ? What do they suggest about the dominant interaction(s) responsible for stabilizing the folded conformation ?

[7]

(c) Describe the equilibrium dialysis method for determining protein-ligand binding affinities. For binding of a ligand (L) to a protein (P) to form a 1:1 complex (PL), show that: $c_p/[PL] = 1 + 1/K[L]$, where K is the equilibrium constant and c_p is the total protein concentration. Explain how this expression is used to analyse equilibrium dialysis data.

[6]

(d) In an equilibrium dialysis experiment to study the binding of a new organic ligand to a soluble receptor protein, the following data were obtained:

Left-hand (protein + ligand) compartment:

Total protein concentration = 8.3×10^{-9} M

Total ligand concentration = 3.9×10^{-8} M

Right-hand (ligand only) compartment:

Total ligand concentration = 3.5×10^{-8} M

What is the equilibrium binding constant for this process ?

[6]