Answer Notes

[Do not consult these until you have attempted the questions.]

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1. (a) Levinthal paradox - too many polypeptide conformers to explore in realistic timescale. Numerical estimate: e.g. assume 3 x 3 = 9 possible Φ-Ψ angles per peptide, leads to typically 100^9 possible conformers for typical protein. Implies that protein folding must follow specific pathways.

(b) (i) Use $\Delta G° = -RT.\ln(K) = \Delta H° - T.\Delta S°$ to complete:-

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	**454.9**
50	**0.345**	2.86	175.0	**532.9**
55	**1**	0	200.0	609.8
60	3.22	**-3.24**	225.0	**685.4**

(ii) Fraction unfolded = K/(1+K) = 0.26, 0.5, 0.76 (respectively)

(iii) Increase in ΔH° with temperature (confirmed by increase in ΔS° with T) signifies a positive heat capacity increment (ΔC_p), characteristic of hydrophobic stabilizing interactions.

(c) Spectroscopic methods (UV, fluorescence, CD)-changes in environment/conformation on binding
Hydrodynamics (viscosity, sedimentation)-changes in gross macromolecular properties
Calorimetry (DSC, ITC)-direct measure of energy changes on binding
Equilibrium dialysis-direct measure of ligand binding

- all covered in lectures. Thermodynamic information may be obtained indirectly from temperature dependence, $\Delta G° = -RT.\ln(K) = \Delta H° - T.\Delta S°$, etc., or directly ($\Delta H°$, ΔC_p) by microcalorimetry.

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2. (a) Standard lecture material. High m.p./b.p., high density of liquid (ice floats), high dielectric, 4°C max density, heat capacity, etc... H-bonding, residual open tetrahedral lattice structure... ∴ hydrogen bonding between biomolecular groups generally unfavourable in water (water mols compete for H-bonding sites), but hydrophobic interactions (arising from structural propoerties of liquid water) more significant..

(b) Use $K = [NMA_2]/[NMA]^2$; $\Delta G° = -RT.\ln(K) = \Delta H° - T.\Delta S°$

Results (for 25 °C):

Solvent	K /M^{-1}	ΔG° /kJ mol^{-1}	ΔH° /kJ mol^{-1}	ΔS° /J K^{-1} mol^{-1}
CCl_4	4.7	- 3.8	-17.6	- 46
Dioxane	0.52	+ 1.6	- 3.3	- 16.5
Water	0.005	+ 13.1	0	- 44

Suggests H-bonding unfavourable in polar solvents.

(c) Anything sensible from: UV difference, fluorescence, CD, nmr, viscosity, hydrodynamic effects, DSC, etc. (all done in lectures). Use $\Delta G^\circ = -RT.\ln(K)$, where $K = [U]/[N] = (F-F_0)/(F_{inf} - F)$, for any observable F.

(d) Positive increase in excess heat capacity of unfolded protein w.r.t. folded. Observed from temperature dependence of ΔH or calorimetric (DSC) studies. Characteristic of hydrophobic contribution to protein folding.

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3. (a) Standard lecture material: High mp/bp, density increase on melting (ice floats), high dielectric, 4 °C max density, high heat capacity... Water dipole, H-bonding, residual open tetrahedral lattice structure ∴hydrogen bonding between biomolecular groups generally unfavourable in water (water molecules compete for H-bonding sites), but hydrophobic interactions (arising from structural properties of liquid water) more significant.

[7]

(b) Partitioning between solvents, gaseous dimers
Liquid phase dimerization of peptide analogues (e.g. N-methyl acetamide)
Different results in aqueous/non-aqueous systems
Polar vs. non-polar environments in folded/unfolded proteins - [lecture material]

[6]

(c) Both F and CD follow the same transition in this case, so may use either.

$T_m = 50$ °C (mid-point of unfolding transition)

Fraction unfolded $= (F - F_0)/(F_{inf} - F_0) = (58.8 - 65)/(15 - 65) = 0.124$

Equilib.const. for unfolding

$K = (F - F_0)/(F_{inf} - F) = (58.8 - 65)/(15 - 58.8)$
$= 0.142$

$\Delta G^\circ_{unf} = -RT.\ln K = -8.314 \times (273 + 46) \times \ln(0.142) = +5.18$ kJ mol^{-1}

[7]

(d) Fluorescence is probing the polarity of the environment of aromatic amino acid residues (primarily tryptophan), which changes (non-polar --> polar) as the protein unfolds. CD measures secondary structure changes (α-helix, β-sheet, etc.). These do not necessarily occur simultaneously, since protein unfolding may take place in two (or more) steps, e.g. change in tertiary structure exposing aromatic groups but retaining secondary structure, followed by "melting" of the secondary structure at higher temperatures.

[5]

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4. (a) Gas phase dimerization, e.g. formic acid
Liquid phase dimerization of peptide analogues (e.g. N-methyl acetamide)
Different results in aqueous/non-aqueous systems - [lecture material]

[8]

(b) Hydrogen bonds required for structure, but do not necessarily contribute to stability, because of competing H-bonds with water. Hydrophobic interactions are most likely source of stability, supported by evidence from thermodynamics (ΔC_p, etc.) - [lecture material]

[5]

(c) Plasticisers are hydrophobic compounds that can mimic endocrines and act as feminizing hormones by binding to receptor and fatty-acid transport proteins - [tutorial material]

[3]

(d) $D = [A]_{water}/[A]_{cyclohexane}$ (or equivalent)

$\Delta G°_{transfer}$(cyclohexane→water) = $-RT.\ln D$ hence D....

n	$\Delta G°_{transfer}$ / kJ mol^{-1}	$D = \exp(-\Delta G°_{transfer} \times 1000/8.314 \times 298)$
2	- 0.2	1.1
4	+ 6.9	0.06
8	+ 21.1	0.0002

[6]

n = 8 probably best since, even though this would probably also act best as an endocrine disrupter, it will have the least propensity to leach into aqueous environment. (Or anything equally sensible, or better !)

[3]

[25 marks total]

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5. (a) Standard lecture material - electrostatics, charge-charge, dipole-dipole, etc., H-bonds, hydrophobic interactions, London dispersion forces. Water can affect indirectly (via dielectric constant effect) or directly (via H-bonding to water mols.). Water is essential for hydrophobic interaction !

(b) For globular protein unfolding:

$\Delta H°_{unf}$ normally positive (endothermic), but temperature dependent (positive ΔC_p)
$\Delta S°_{unf}$ normally positive (favourable), again depends on T.
$\Delta G°_{unf}$ positive below T_m , negative above T_m , $T_m = \Delta H° / \Delta S°$
Need to overcome large configurational entropy of unfolded polypeptide chain to stabilise folded conformation.
$\Delta G°$ per residue much less than RT, therefore folding must be cooperative.

Positive heat capacity increment (ΔC_p) conventionally taken to be characteristic of hydrophobic interactions (from small model compounds).
Determined by microcalorimetry or temperature dependence of spectroscopic properties, etc.

(c) Equilibrium dialysis (from lectures) - two compartments, semi-permeable membrane, protein/macromolecule confined to one side, (small) ligand free to move across membrane so that, at equilibrium, measurements of total protein and total ligand either side of membrane will give all info necessary to determine K. Appropriate sketch diagram.

$$P + L \rightleftharpoons PL$$

$c_p = [PL] + [P]$; $K = [PL]/[P][L]$

$\therefore\ c_p/[PL] = 1 + [P]/[PL] = 1 + 1/K[L]$ (QED)

Slope of d-r plot = $1/Kc_p$. Useful because only equilibrium dialysis (and related methods) gives free ligand concentration [L] directly. For most other methods need to make approximations, or fit to complete binding expression.

(d) c_p = [PL] + [P] = 8.3×10^{-9} M (from left hand compartment)
c_L = [PL] + [L] = 3.9×10^{-8} M .. ..

Free ligand [L] = 3.5×10^{-8} M (from right hand compartment)

$\therefore$ [PL] = 3.9×10^{-8} - 3.5×10^{-8} = 4.0×10^{-9} M

[P] = 8.3×10^{-9} - 4.0×10^{-9} = 4.3×10^{-9} M

Hence K = [PL]/[P][L] = 2.7×10^{7} M^{-1}

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6. (a) Spectroscopic methods (UV, fluorescence, CD)
-changes in environment/conformation on folding or binding
Hydrodynamics (viscosity, sedimentation)
-changes in gross macromolecular properties
Calorimetry (DSC, ITC)
-direct measure of energy changes on unfolding or binding
Equilibrium dialysis
-direct measure of ligand binding

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(b) (i) Use $\Delta G^\circ = -RT.\ln(K) = \Delta H^\circ - T.\Delta S^\circ$ to complete:-

t /°C	*K*	ΔG° /kJ mol^{-1}	ΔH° /kJ mol^{-1}	ΔS° /J K^{-1} mol^{-1}
35	0.28	3.26	75.0	<u>232.8</u>
40	<u>0.46</u>	2.02	100.0	<u>312.9</u>
45	<u>1.0</u>	0	125.0	392.9
50	2.85	<u>-2.81</u>	150.0	<u>472.9</u>

(ii) Fraction unfolded = K/(1+K) = 0.22, 0.5, 0.74 (respectively)

(iii) Increase in ΔH° with temperature (confirmed by increase in ΔS° with T) signifies a positive heat capacity increment (ΔC_p), characteristic of <u>hydrophobic stabilizing</u> interactions.

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