BIOPHYSICAL CHEMISTRY (CY60004) CLASS TEST

Q1. (a) Draw a dipeptide comprised of Ser-Phe at physiological pH. Mark the ϕ, ψ and ω angles. [3]

(b) An unspecified protein contains a 15-residue long α-helix with the following sequence:
WEANIKQRLSTVYKQ [3]

(i) How many full turns are in this α-helix?

(ii) What is the length of the helix (in Angströms) in the direction of the helix axis?

(iii) How many hydrogen bonds between the backbone atoms are in this helix?

(c) Write the sequence of the peptide Cys-His-Glu-Met-Ile-Ser-Thr-Arg-Tyr-Lys-Gly-Pro using the one letter code for amino acids. [4]

Q2. (a) List three forces or kinds of interactions that influence protein structure. For each, indicate whether it is favorable or unfavorable, and whether its primary effect is enthalpic or entropic. [3]

(b) Name three major factors that influence the melting temperature (T_m) of DNA. [3]

(c) How much iron in grams is contained the average person's hemoglobin. The following information may be required: Molecular weight of hemoglobin 64 kDa; Atomic weight of iron 56 gm/mol; number of red blood cells in human blood 2.5 x 10^{18}; volume of blood 7 litres; concentration of hemoglobin in blood 0.16 gm/ml. [3]

Q3. (a) What are the different ways in which a bisubstrate mechanism can occur? Illustrate your answer with a schematic representation for the formation of two products from two substrates. [3]

Q4. (a) Proteins P-T show the following pI values: P=4.15, Q=6.20, R=7.50, S=7.50, T=8.95. If a mixture of these proteins at pH 7.50 is added to an anion exchange column which protein will elute first? If the column were changed to a cation exchange column, which protein would elute first? It was observed that Protein R eluted faster than Protein S from an anion exchange column. Suggest a possible reason for this. [3]

(b) In the Table, information about 8 proteins has been provided. On a sketch of a 2D gel diagram show the positions of the proteins after isoelectric focusing and SDS-PAGE. Label the diagram clearly. [4]

	Monomer MW (kDa)	pI	Oligomer state
A	14	5.5	dimer
B	19	6.0	monomer
C	24	8.0	monomer
D	29	5.2	monomer
E	29	9.5	dimer
F	35	4.3	monomer
G	47	6.5	tetramer
H	47	7.5	dimer

Q5. (a) Some lipoproteins sediment in a centrifugal field while others are found to float. What is the partial specific volume of a lipoprotein that neither sinks nor floats in a solution of density 1.125 gm/cm^3? [3]

(b) (i) Carbonyl hemoglobin is found to move from 4.525 cm to 4.599 cm in 30 minutes at a rotor speed of 39,300 rpm. Calculate the sedimentation coefficient s in Svedbergs.

(ii) At 303 K the diffusion coefficient of carbonyl hemoglobin is D=7x10^{-7} cm^2 sec^{-1}. The specific volume of carbonyl hemoglobin is 0.755cm^3/gm and the solvent density ρ is 0.9469 gm/cm^3. Calculate the molecular weight of carbonyl hemoglobin. [5]

(c) What is the second virial coefficient? How is it determined? [3]

$\overset{CH_2OH}{NH_2 - CH - COOH}$ | $\overset{CH_2-Ph}{NH_2 - CH - COOH}$

ser phe

1/2 1/2 1/2

Q1. (a) Draw a dipeptide comprised of Ser-Phe at physiological pH. Mark the ϕ, ψ and ω angles. [3]

$H_3\overset{+}{N} - COO^- - 1$

Ser-Phe $- 1/2$

$C_\alpha - C \rightarrow \psi$
$C_\alpha - N \rightarrow \phi$.

$C - C \rightarrow \psi$
peptide $C - N \rightarrow \omega$
$N - C - \phi$

(b) An unspecified protein contains a 15-residue long α-helix with the following sequence: **WEANIKQRLSTVYKQ** [3]

(i) How many full turns are in this α-helix? 1

One turn contains 3.6 residues; therefore 15 residues form 4 full turns (15/3.6 = 4.2).

(ii) What is the length of the helix (in Angströms) in the direction of the helix axis? 1

The length of one turn is 5.4 A, so a 15-residues α-helix will be 15/3.6 x 5.4 A = 22.5 A long.

(iii) How many hydrogen bonds between the backbone atoms are in this helix? 1

1 residue can be involved in max 2 H-bonds, therefore 15 residues can make up to 2x15=30 H-bonds.

In the α-helix, 4 residues at the N-terminus and 4 at the C-terminus make only 1 bond per residue.

This makes the total number of H-bonds 30-2x4=22. When calculating this number, each H-bond was counted twice: one time for the donor residue and one time for the acceptor. The real number of H-bonds is then 22/2=11.

(c) Write the sequence of the peptide Cys-His-Glu-Met-Ile-Ser-Thr-Arg-Tyr-Lys-Gly-Pro using the one letter code for amino acids. [4]

↓ E ↓ R ↓ Y ↓ K

C-H-E-M-IS-T-R-Y-K-G-P

Q2. (a) List three forces or kinds of interactions that influence protein structure. For each, indicate whether it is favorable or unfavorable, and whether its primary effect is enthalpic or entropic. [3]

Hydrophobic interactions; release of water and burial of non-polar groups. Favorable. ΔS.
Electrostatic interactions: H-bonding, ionic interactions,between backbone or sidechains. Slightly favorable or neutral (because the same interactions can form with water in unfolded state). ΔH.
Disulfide bonds, metal ion binding. Favorable. ΔH, but pay a cost in ΔS.

(b) Name three major factors that influence the melting temperature (T_m) of DNA. [3]
1. GC content: the higher the GC%, the higher the T_m
(G-C base pairs have 3 H-bonds and are thus stronger than A-T base pairs)
2. Salt: the higher the [salt], the higher the T_m
(ions shield charges and thus lessen repulsion between phosphates)
3. Low (<2.3) or high (>11.5) pH decrease T_m
(ionization of the bases)
4. Organic compounds that destabilize the double helix by competing as H-bond partners or by disrupting the water clathrate shell around the bases

(b) (i) Carbonyl hemoglobin is found to move from 4.525 cm to 4.599 cm in 30 minutes at a rotor speed of 39,300 rpm. Calculate the sedimentation coefficient s in Svedbergs.
(ii) At 303 K the diffusion coefficient of carbonyl hemoglobin is D=7x10^{-7} cm^2 sec^{-1}.
The specific volume of carbonyl hemoglobin is 0.755cm^3/gm and the solvent density ρis 0.9469 gm/cm^3. Calculate the molecular weight of carbonyl hemoglobin. [5]

b(i) ~~(a)~~ Carbonyl hemoglobin is found to move from 4.525 cm to 4.599 cm in 30 minutes at a rotor speed of 39,300 rpm. Calculate the sedimentation coefficient s.

Solution:

$$\omega = 2\pi(39{,}300\,\text{min}^{-1})(\text{min}/60\,\text{sec}) = 4113\,\text{sec}^{-1}$$

1 $$s = \frac{1}{\omega^2}\frac{d\ln r}{dt} \approx \frac{1}{\omega^2}\frac{\Delta \ln r}{\Delta t} = \frac{1}{(4113)^2}\frac{\ln(4.599) - \ln(4.525)}{(30)(60)} = \frac{\ln(4.599/4.525)}{3.05\times 10^{10}}$$ 1

Computing the result: $s = \dfrac{1.59\times 10^{-2}}{3.05\times 10^{10}} = 5.21\times 10^{-13}$ sec. 1

b(ii) ~~(b)~~ At 303 K the diffusion coefficient of carbonyl hemoglobin is D=7x10^{-7} cm^2 sec^{-1}. The specific volume of carbonyl hemoglobin is $\bar{v}_2 = 0.755 cm^3/gm$ and the solvent density is ρ=0.9469 gm/cm^3. Calculate the molecular weight of carbonyl hemoglobin.

Solution:

1 $$M = \frac{RTs}{D(1-\bar{v}_2\rho)} = \frac{(8.31\times 10^7)(303)(5.21\times 10^{-13})}{(7\times 10^{-7})(1-(0.755)(0.9469))} = 65{,}846\,gm/mole$$ 1

X (c) Calculate the coefficient of friction of carbonyl hemoglobin at T=303K.

Solution: $$f = \frac{k_B T}{D} = \frac{(1.38\times 10^{-23}\,J/K)(303K)}{7\times 10^{-7}\ \text{cm}^2/\text{sec}} \times \left(\frac{10^4\,cm^2}{m^2}\right) = 59.7\times 10^{-12}\,kg/s$$

(c) What is the second virial coefficient? How is it determined? [3]

$$\frac{Kc}{R_\theta} = \frac{1}{M_w} + 2B_2c,$$ (1)

where the optical constant $K = [2\pi n(dn/dc)]^2/N_A\lambda_o^4$. R_θ is the excess Rayleigh ratio, M_w is the weight average molecular weight of the solute, c is the concentration (in g/ml), B_2 is the second virial coefficient, λ_o is the wavelength of light used, N_A is Avogadro's number, and dn/dc is the refractive index increment due to the solute under a given set of solution conditions. A positive B_2 value indicates repulsion between molecules whereas a negative B_2 indicates the presence of intermolecular attraction [2]. However, in multicomponent systems,

Measure of non-ideality 1

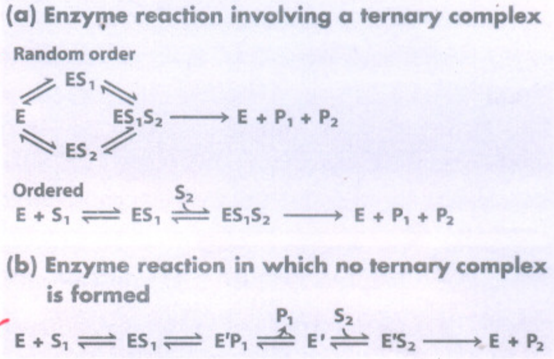
Plot of $\frac{Kc}{R_\theta}$ vs c slope $2B_2$

(c) How much iron in grams is contained the average person's hemoglobin. The following information may be required: Molecular weight of hemoglobin 64 kDa; Atomic weight of iron 56 gm/mol; number of red blood cells in human blood 2.5×10^{18}; volume of blood 7 litres; concentration of hemoglobin in blood 0.16 gm/ml. [3]

0.16 g/ml x 7000ml = 1120g i.e. 1120/64000 = 0.0175 moles 2

4 Fe bound for each mole of Hb : means 0.0175 x 56g Fe = 3.92g Fe 1

Q3(a) What are the different ways in which a bisubstrate mechanism can occur? Illustrate your answer with a schematic representation for the formation of two products from two substrates. [3]



Sequential binding of S1 and S2 before catalysis:
Random substrate binding - Either S1 or S2 can bind first, then the other binds.
Ordered substrate binding - S1 must bind before S2.

Ping Pong reaction - first S1 → P1, P1 released before S2 binds, then S2 → P2.

Q4(a) Proteins P-T show the following pI values: P=4.15, Q=6.20, R=7.50, S=7.50, T=8.95. If a mixture of these proteins at pH 7.50 is added to an anion exchange column which protein will elute first? If the column were changed to a cation exchange column, which protein would elute first? It was observed that Protein R eluted faster than Protein S from an anion exchange column. Suggest a possible reason for this. [3]

Protein to elute fastest on an anion exchange T 1
Protein to elute fastest on a cation exchange P 1

Both proteins have zero net charge – may be charge distribution is different 1

Q5. (a) Some lipoproteins sediment in a centrifugal field while others are found to float. What is the partial specific volume of a lipoprotein that neither sinks nor floats in a solution of density 1.125 gm/cm^3? [3]

$$s = \frac{M(1-\overline{v_2}\rho_0)}{Nf} = \frac{v}{\omega^2 r}$$ 1

If the lipoprotein does not sink or float this implies the velocity v=0. This is possible only if

$$(1-\overline{v_2}\rho_0) = 0 \therefore \overline{v_2}\rho_0 = 1 \; and \; \overline{v_2} = \frac{1}{\rho_0} = \frac{1}{1.125\, gcc^{-1}} = 0.8889\, cc/g$$ 1

1

4(b) In the Table, information about 8 proteins has been provided. On a sketch of a 2D gel diagram show the positions of the proteins after isoelectric focusing and SDS-PAGE. Label the diagram clearly. [4]

	Monomer MW (kDa)	pI	Oligomer state
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Labels important

½ marks for location of band

Remember in an SDS PAGE monomer molecular weight important