Fall '13 | CH370 | Name KEY
Hackert | Exam II | UTeID

$R = 8.314 \times 10^7$ g-cm^2/(sec^2-mol-K); RCF = $(1.119 \times 10^{-5})(\text{rpm})^2(\text{r})$; $s = v/\omega^2 r$
$\rho_{water} = 1.00$ g/cm^3; $s = M(1 - \nu'\rho)/N^o f$; $(1/c_r)(dc_r/dr) = M\omega^2 r(1 - \nu'\rho)/RT$;
$A = A_o \exp(-kt)$; $k = 1.38 \times 10^{-23}$ J/K; $h = 6.63 \times 10^{-34}$ J-sec; $KC/R_\theta = 1/(M*P(\theta)) + 2A_2C$
$\Delta c = (\Delta J \times \lambda)/(a \times K)$; $\eta = 0.01$ g/(cm-sec); $N^o = 6.02 \times 10^{23}$ mol^{-1}; $k = 1.38 \times 10^{-23}$ J/K;
$|E(1\%) \times MW| = |10 \times \varepsilon|$; Eff. = $1/(1 + (R/Ro)^6)$;
(Note: Set up equations and show work to get full or partial credit on all calculations.)

1. Spectroscopy: At 280 nm the molar extinction coefficients of tryptophan is $\varepsilon = 5.6 \times 10^3$ M^{-1}cm^{-1} and tyrosine is $\varepsilon = 1.4 \times 10^3$ M^{-1}cm^{-1}. A protein has a molecular weight of 176,548 g/mol and a molar extinction coefficient of 157,125 M^{-1}cm^{-1} at 280 nm. A sample of the protein in a 0.50 cm cuvette was found to have a T = 0.32 at a wavelength of 280 nm. (Show work for credit. Draw a box around your answers.)

A) What is the **absorbance** for this sample protein solution?

(3) $A = \log\left(\frac{1}{T}\right) = -\log T = 0.495$ (3) (2)

B) What is the E(1%) of this protein? Units (1) 1 - units

(2) $E^{1\%} = \left|\frac{10 \cdot \varepsilon}{M}\right| = \left|\frac{10 \times 157{,}125}{176{,}548}\right| = 8.90\left(\frac{1}{cm \cdot (g/dL)}\right)$

C) Calculate the concentration of this protein solution in **molarity**.

(3) $A = \varepsilon \cdot [\,] \cdot l \Rightarrow [M] = \frac{A}{\varepsilon \cdot l} = \frac{0.495}{(157{,}125\ M^{-1}cm^{-1})(0.50\,cm)}$

$[\,] = 6.3 \cdot 10^{-6} M = 6.3\,\mu M$ (1)

D) Calculate the concentration of this protein solution in **mg/mL**.

(3) $A = E^{1\%} \cdot c \cdot l$ $\quad c(g/dL) = \frac{A}{E^{1\%} \cdot l} = \frac{0.495}{8.9\left(\frac{1}{cm}\right)\left(\frac{1}{g/dL}\right) \cdot (0.50\,cm)}$ (1)

$c(g/dL) = 0.111\,g/dL$ (1) $\left(\frac{10\,dL}{L}\right)\left(\frac{1\,L}{1000\,mL}\right)\left(\frac{1000\,mg}{g}\right)$

$c = 1.11$ mg/mL (1)

2. FRET: You have available a pair of chromophores, Halloween Orange-31 and Mellow Yellow-22, that are known to have strong spectral overlap with a $R_o = 38.0$ Å (3.80 nm) for use in a FRET experiment to measure separation distance of two ligand binding sites. The measured efficiency of energy transfer is 38%. What is the estimated distance between binding sites? 41.2 Å
(Show Work for credit, show units.)

(6) $Eff = 0.38 = \frac{1}{(1 + (r/r_o)^6)}$ (1) $\rightarrow$ let $x = \frac{r}{r_o} \rightarrow$

$0.38 + 0.38x^6 = 1$

$0.38x^6 = 0.62 \Rightarrow x^6 = 1.631$

$x = 1.085 \Rightarrow r = 41.2$ Å

$(1.085 \cdot 38)$

3. **Radioactivity:** Consider an isotope "X" with 83 protons and 103 neutrons.

(2) **A)** What is the element and isotope corresponding to X? $^{186}_{83}Bi$ (1) (1)

Identify the **isotope** produced by the following events:

(2/3) **B)** $X \rightarrow {}^{186}_{82}Pb$ (1) (1) $+ {}^{0}_{+1}$ beta(+) ; $X + EC \rightarrow {}^{182}_{80}Hg$ (1) (2) $+ {}^{4}_{2}$ alpha

39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
71 Lu	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn

C) A radioisotope has a **rate constant** of **0.10 / day.** (4)

i) Calculate the **half-life** of the radioisotope. **Half-life =** 6.93 days (with units)

Show work here:

(4) $A = A_0 e^{-kt} \Rightarrow \ln \frac{A_0}{A} = \ln 2 = k t_{1/2}$ (1) or $t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{0.1\ d^{-1}}$

ii) How many **days** will it take for a sample of this radioisotope initially at 256 mCuries to decrease to 1 mCurie of radioactivity? 55.5 days **days.** Show work here:

(4) $A = A_0 e^{-kt} \Rightarrow \ln \frac{A_0}{A} = kt \Rightarrow \frac{\ln 256}{0.10/d} = t = 55.5$ days (4)

(2)

(or 8 half-lives)

D) Detector Matching (G = Geiger; LSC = liquid scintillation counting; IP = image plate)

(3) i) best dynamic range IP (1) (Ans. may be used more than once)

ii) uses photomultiplier tubes LSC (1)

iii) primary and secondary fluors LSC (1)

4. SDS-PAGE:

A) What are the purpose and characteristics of the stacking gel in DISC gel PAGE?

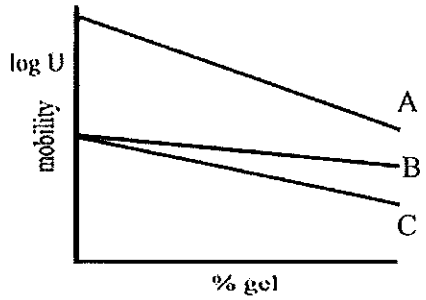
(4) Stacking gel - prepares sample to enter running gel. In DISC gel PAGE, stacking gel lower % gel (2) and lower pH (2) so glycine in buffer ~0 charge ⇒ proteins to carry current.

B) Most proteins take up Coomassie blue stain proportional to the amount of protein in the band. What can you conclude about the subunit composition of a protein you isolated that gave the following experimental results: SDS PAGE shows three bands running at 30 kDa (α band), 60 kDa (β band) and 120 kDa (γ band) with all three bands integrating to nearly identical stain density.

(3) Subunit composition (e.g. α2β2γ2): $(\alpha_4 \beta_2 \gamma_1)$ (1) (1) (1)

α 4×30 = 120
β 2×60 = 120
γ 1×120 = 120

5. Plots: Consider proteins A, B, and C that exhibit the mobility behavior shown at right, which protein can you conclude is the largest and which protein carries the least total charge under the run conditions?



(2/2)

Largest: A (2) (steepest slope)

Least total charge: B (2) (smallest & low mobility)

6. 2D Electrophoresis: You performed a 2D IEF-SDS PAGE experiment on peptides P1, P2, P3, and P4 and obtained the following results. Match each spot with its peptide number.

A = P1; B = P4; C = P3; D = P2.

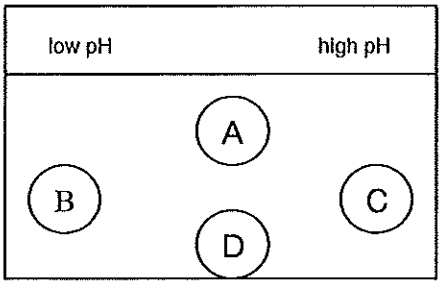
(4)

1 each P1= FAGRRALVEDPIW ~0

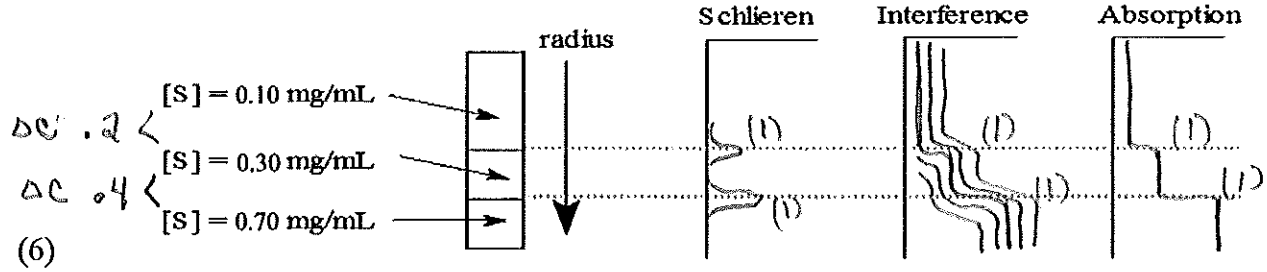
P2= RACKED ~0

P3= IKLRGAKPV (+)

P4= AGWDPLEFD (−)



7. Optics: Consider the following low-speed, diffusion run looking at an ultracentrifuge sample cell with three solutions layered over each other at the concentrations shown on the left. On the right, sketch the appearance expected for Schlieren, Interference and Absorption optics results expected as a function of "r" or position in the cell.



(6)

8. Analytical Ultracentrifugation: $s = \frac{M(1-\bar{v}\rho)}{N_0 f}$

A) Name the two molecular factors that influence the value of its sedimentation coefficient, **s.**

(2) M (Molecular Wt.) (1) f (Frictional coef.) (1) $\bar{v}$

B) Sedimentation velocity: The sedimentation coefficient (s) of a protein is suspected to be around 8S. Calculate the rotor speed in rpm to use for a sedimentation velocity run if we would like the boundary to move from r_1 = 6.72 cm to r_2 = 7.72 cm over a run time interval of 60 min.

(Assume: $\rho_{protein}$ = 1.32 g/cm^3; ρ_{solv} = 1.00 g/cm^3; v_{bar} = 0.72 cm^3/g; T = 20°C)

Rotor speed (rpm) = 66275 rpm

(6)

$\ln r_2/r_1 = s\omega^2\Delta t \Rightarrow \omega^2 = \frac{\ln(7.72/6.72)}{(2)(8\cdot10^{-13}\,sec)(3600\,sec)}$ (1)

$\omega^2 = 4.82\cdot10^7/sec^2$ (1)

$\omega = 6940/sec\left(\frac{60\,sec}{min}\right)\left(\frac{1\,rev}{2\pi}\right) = 66275$ rpm (6)

C) Concentration by Interference Optics: Consider a centrifuge double sector cell with protein solution on the sample side and dilute buffer on the reference side that has a hairline crack between the two sectors so that the buffer can layer over the protein solution once the centrifuge is turned on. For this experiment, the T = 20°C, the rotor speed w = 5500 rpm, the radius to the sample optics section is 7.10 cm, the cell path length to be 12.00 mm, λ = 546 nm, and (dn/dc = 0.186 $(g/cm^3)^{-1}$. Using interference optics to monitor the run, the results show a no displacement from 7.10 to 7.25 cm with a total displacement of 4.75 interference fringes at 7.60 cm in the cell. Estimate the concentration of the protein sample in mg/mL at 7.60 cm.

Place answer here 1.16 mg/mL What assumption(s) did you have to make?

(6)

$$C \approx 0 + \Delta C = \frac{\Delta J \cdot \lambda}{a \cdot K} = \frac{(4.75)(546\,nm)}{(12 \cdot 10^6\,nm)(0.186\,g/cm^3)}$$

$$C = 0.00116\ g/cm^3 \times \left(\frac{1000\,mg}{g}\right)$$

(1) (2)

$$C = 1.16\ mg/mL$$ (1)

Had to assume $C \approx \Delta C$ on basis of no displacement from 7.10 to 7.25 cm.

D) You have sequenced the gene of your favorite protein, and you know it is composed of subunits containing 377 amino acid residues for a subunit molecular weight of 41,234 g/mol. Your protein comes off in the void volume of a G-150 column and you suspect the protein is either a dodecamer (12 subunits) or an octamer (8 subunits). To determine the oligomeric nature of your protein you are scheduled to do a **sedimentation equilibrium** run over the weekend. The temperature of the system will be maintained constant at 20 °C. The density of your protein is estimated by its amino acid composition to be 1.36 g/mL and its v-bar estimated to be 0.735 cm^3/g. The density of the solvent solution is 1.02 g/mL. To get the best data from the experiment, you would like to have 7 times the concentration at r_2 (7.60 cm) than at your reference point r_1 (7.00 cm). **Assuming your protein to be an octamer, calculate the rotor speed in rpm** that you should run your sedimentation equilibrium experiment to achieve the **7x difference in concentration between r_1 and r_2.**

(8)

Submit $M = 41,234$; $M_8 = 329,872\ g/mol$ (1)

$$\ln \frac{c_2}{c_1} = \frac{M(1-\bar{v}\rho)}{2RT}(r_2^2 - r_1^2)\omega^2$$

$R = 8.314 \cdot 10^7\ \frac{g \cdot cm^2}{s^2 \cdot m \cdot K}$ (1)

$T = 293\,K$

$r_2^2 - r_1^2 = 8.76\ cm^2$ (1)

(3)

$$\omega^2 = \frac{\ln(7)\,2\,(8.314 \cdot 10^7\ g \cdot cm^2/s^2 \cdot m \cdot K)(293\,K)}{(329{,}872\ g/m)(1 - 0.735(1.02))(8.76\ cm^2)} = 131{,}232\ /sec^2$$

(.25) (1)

$$\omega = 362\ /sec \times \left(\frac{60\,s}{min}\right)\left(\frac{1\,rev}{2\pi}\right) = 3459\ rpm$$ (1) (8)

9. Consider a properly folded, 150 kDa globular protein. Estimate both the **value and units** of "s" and the "radius" (*within one order of magnitude*), and just the **units** of "D" expected for this protein.

(5)

a) s ~ $9.8\,S = 9.8 \cdot 10^{-13}\,s$ (1) (1) b) radius 3.5 Å (1) (1)^4 c) units on D cm^2/sec (1) $(6 \cdot 10^{-7})$

10. Light Scattering:

A) If the scattering signal at wavelength (λ) is represented by "R", what describes the scattering at wavelength (2λ)?

(2) i) 2R ii) 4R iii) 8R iv) 16R v) R/2 vi) R/4 vii) R/8 (vii) R/16

B) By measuring the angular dependence of "static" light scattering of large molecules, we can obtain two important properties of the large molecule, namely:

(2) Molec. Wt. and r_g (radius of gyration)

C) What is the most common result reported from a "dynamic" light scattering experiment?

(2) R_h (hydrodynamic radius) { Meas. fluctuations on short time scale → 0 → f → R_h

D) A sample that appears to be pure on SDS PAGE was analyzed by static light scattering (LS) and refractive index increment (RI) measurements and found to have a major peak in each with a 2:1 ratio, but also a minor peak at a higher molecular weight with a 6:1 ratio. What might be an explanation for the appearance of the "contaminant" peak seen in the LS and RI measurements?

(3) $LS \propto []\cdot M$ } → The LS signal of a Trimer will be 3X
$RI \propto [\,]$ } enhanced relative to monomer,
→ Presence of Trimers { Monomer (2:1) → would expect Trimer (6:1)

E) Why is light scattering a particularly good method for checking for high molecular contaminants in solution samples?

(3) $LS \propto [\,]\cdot M$; very small % of a high molecular contaminants give rise to a relatively large signal. (e.g. 1% of a $M \sim 10^6$ contaminant would have same LS signal as the 99% ($M \sim 10^4$))

11. CD:

A) What is the basis for CD measurements and what special instrument needs are necessary to use CD to study protein solutions?

(3) CD measures the difference in absorbance between L- and R-circularly polarized light. Need special lamps and cuvettes to work in UV (~200nm) range; generate cir. polarized light; meas. small values as difference of large numbers (sens.)

B) Two molecules are found that absorb light at the same wavelength and to the same extent. They also exhibit the same CD intensity profile except Molecule A has a positive CD and Molecule B has a negative CD. What is the structural relationship between Molecule A and Molecule B?

(2) Optical isomers (enantiomers)

(Please sign your name on the back near the top of this exam in a manner that you can recognize for returning it to you)