Fall '14 BCH370 Name KEY
Hackert Exam II UTeID

$R = 8.314 \times 10^7$ g-cm^2 /(sec^2-mol-K); RCF = (1.119×10^{-5})(rpm)^2(r); Eff. = 1 / (1 + (R/Ro)^6)

$\rho_{water} = 1.00$ g/cm^3; $s = M(1 - \nu'\rho)/N^o f$; $(1/c_r)(dc_r/dr) = M\omega^2 r(1 - \nu'\rho)/RT$; $s = v/\omega^2 r$

$A = A_o \exp(-kt)$; $k = 1.38 \times 10^{-23}$ 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;

(Note: Set up equations and show work to get full or partial credit on all calculations.)

1. FRET: You have available a pair of chromophores, Halloween Orange-31 and Red Raider-1, that are known to have strong spectral overlap with a $R_o = 47.0$ Å (4.70 nm) for use in a FRET experiment to measure separation distance of two ligand binding sites. The measured efficiency of energy transfer is 33%. What is the estimated distance between binding sites?

$r = 52.9$ Å (Show Work for credit, show units.)

(6) $Eff = 0.33 = \frac{1}{(1+(\frac{r}{r_o})^6)}$; let $X = \frac{r}{r_o}$, then $0.33 = \frac{1}{1+X^6} \rightarrow$

$0.33 + .33X^6 = 1 \rightarrow X^6 = \frac{0.67}{0.33} \rightarrow X^6 = 2.03$ or $X = 1.125$

$r = 1.125 \cdot r_o$

2. Radioactivity: Consider an isotope "X" with 76 protons and 100 neutrons.

(1) a) What is the element? ^{176}Os (1)

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

(6) b) X $\xrightarrow{(1)}$ ^{176}Ir (1) + beta(-); X $\xrightarrow{(1)}$ ^{172}W (1) + alpha; X + EC $\rightarrow$ ^{176}Re (1)

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 0.020 / yr.

i) Calculate the **half-life** of the radioisotope. **Half-life =** 34.65 yr

Show work here:

(4) $t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{0.020/yr} = 34.65$ yr (1)

ii) How many **years** will it take for 99.9% of a sample of this radioisotope to undergo radioactive decay ? 345 yr. **years.** Show work here:

(4) $A = A_o e^{-kt}$ or $\frac{0.001}{1} = e^{-kt}$ or $\ln(1000) = kt$

d) We discussed several types of detectors used to measure radioactive decay. Briefly describe the principle of the Geiger counter and type of particles best detected using this method.

(4) A metal tube filled with an inert (1) gas (Ar or Ne) at low pressure has a wire (anode) at high voltage (1) relative to metal tube. When ionizing radiation that can penetrate tube (high energy β) enters gas it creates (1) ions which are accelerated to poles (1) creating additional ions and generating a "pulse" to be counted.

3. SDS-PAGE:

a) What would be the impact of forgetting to add the bromophenol blue when doing your SDS PAGE experiment?

(3) No tracking dye and thus would not be able to know when the leading edge was reaching the bottom of gel.

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 40 kDa (α band), 60 kDa (β band) and 120 kDa (γ band) with all three bands integrating to nearly identical stain density.

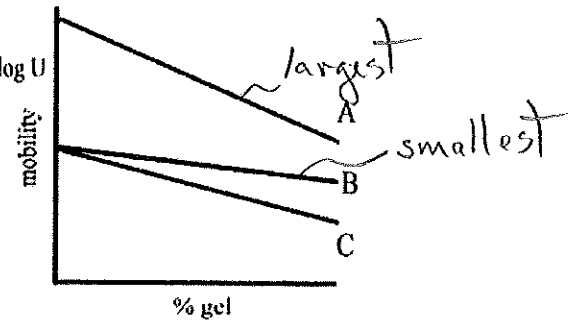
(4) Subunit composition (e.g. α2β2γ2): $\alpha_{3}\beta_{2}\gamma$ (1) (1) (1).

Assume γ = 1 (120 kDa); Then for same amount of protein → 2β and 3α (2×60 = 120; 3×40 = 120)

4. Plots: Consider proteins A, B, and C that exhibit the mobility behavior shown below, which protein can you conclude is the smallest and which protein carries the most total charge under the run conditions?

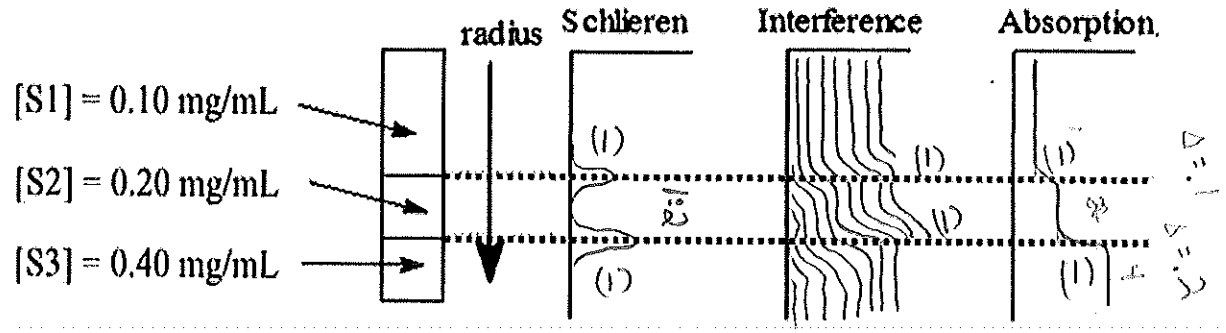
Smallest: B (2)

Most total charge: A (2)



(2/2)

5. Optics: Consider the following low-speed, diffusion run using an ultracentrifuge sample cell with three solutions layered over each other at the concentrations given on the left (Note: Radius increases as shown.) On three frames on the right, sketch the appearance expected for Schlieren, Interference and Absorption optics results expected as a function of "r".



(6)

6. Analytical Ultracentrifugation:

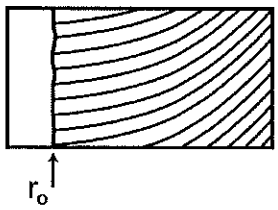
A) Is it possible to measure molecular weight of a macromolecule without running a sedimentation equilibrium experiment? If so, how?

OK, to use to run ultracent. method if correct.

(4) Yes. $s = \frac{M(1-\bar{v}\rho)}{N^\circ \cdot f}$ and $D = \frac{R \cdot T}{N^\circ \cdot f}$ Both "s" and "D" can be measured with anal. ultracentrifuge. Substitute for "f" using D relationship → $M = \frac{s \cdot N^\circ \cdot f}{(1-\bar{v}\rho)} = \frac{s \cdot R \cdot T}{D(1-\bar{v}\rho)}$

B) Sedimentation Equilibrium:

i) You ran a sedimentation equilibrium run employing interference optics during your assigned slot on the instrument over the Halloween weekend. You decided to party rather than monitor the run, so you assumed 4000 rpm would work and you let it run over the weekend to reach equilibrium. On Monday you came in to remove your sample and later noticed that the interference optics for your run looked as shown below. Should you be concerned? If so, why?



(4) Yes, the sample concentration is not zero at r_o, thus $\Delta c \neq c$. The rotor speed should have been slightly higher to deplete sample at meniscus. Cannot est. c since $c_o \neq 0$,

iii) You have sequenced the gene of your favorite protein, and you know it is composed of subunits containing 420 amino acid residues for a subunit molecular weight of 46,754 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 8 times the concentration at r_2 (7.65 cm) than at your reference point r_1 (7.15 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 **8x difference in concentration between r_1 and r_2.** Ans: 3652 rpm (show work for credit)

Submit M = 46,754 g/m → Dodecamer = 12(46,754) = 573,048

(8) Octamer = 8(46,754) = 374,032

$$\ln\left(\frac{c_2}{c_1}\right) = \frac{M(1-\bar{v}\rho)\omega^2}{2 \cdot R \cdot T}(r_2^2 - r_1^2)$$

$M = 374{,}032\ g/m$ (1)
$\bar{v} = 0.735\ cm^3/g$; $\rho = 1.02\ g/ml$
$T = 293K$
$r_2^2 = 7.65^2 = 58.52$; $r_1^2 = 7.15^2 = 51.12$ } $\Delta r^2 = 7.40\ cm^2$ (1)
$R = 8.314 \cdot 10^7 \frac{g\text{-}cm^2}{s^2 \cdot m \cdot K}$ (1)

-2 for no "2"

-2 for calc. mistakes

$$\omega^2 = \frac{\ln(8) \cdot 2 \cdot (8.314 \cdot 10^7 \frac{g\text{-}cm^2}{s^2 \cdot m \cdot K})(293K)}{(374{,}032\ g/m)(1-.735(1.02))\ 7.40\ cm^2}$$

$1-.735(1.02) = 0.2503$

$\omega^2 = 146{,}236\ s^{-2}$

$\omega = 382\ s^{-1}$ or $3652\ rpm$

(-1 no rpm)

7. D and f: Calculate the frictional coefficient and diffusion constant of a protein whose molecular weight is reported in the literature to be 75,000 and its sedimentation coefficient is measured to be 7.2S. (show work for credit)

(Assume: $\rho_{protein} = 1.32$ g/cm^3; $\rho_{solv} = 1.00$ g/cm^3; $v_{bar} = 0.72$ cm^3/g; T = 20°C) ; $s = 7.2 \cdot 10^{-13}$ s

a) **f:** Ans: $4.8 \cdot 10^{-8}$ g/s

(4)

$$s = \frac{M(1-\bar{v}\rho)}{N^\circ \cdot f} \quad \text{or} \quad \boxed{f = \frac{M(1-\bar{v}\rho)}{N^\circ \cdot s}}$$

$$f = \frac{(75000\,g/m)(1-0.72(1.00))}{(6.02\cdot10^{23}/m)(7.2\cdot10^{-13}\,s)} = 4.8\cdot10^{-8}\,g/s$$

b) **D:** Ans: $8.4 \cdot 10^{-7}$ cm^2/s

(4)

$$D = \frac{R \cdot T}{N^\circ \cdot f} = \frac{8.314\cdot10^7 \frac{g\cdot cm^2}{s^2\cdot m\cdot K}(293K)}{6.02\cdot10^{23}/m\,(4.8\cdot10^{-8}g/s)}$$

$$D = 8.4\cdot10^{-7}\,\frac{cm^2}{s} \quad \left(\text{Also, } \frac{s}{D} = \frac{M(1-\bar{v}\rho)}{RT}\right)$$

8. 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) $1/\lambda^4$

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) M (1) and R_G (radius of gyration) (1)

C) 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) $LS \propto M \cdot c$, $RI \propto c$ } IF the major peak where normalized 1:1, then minor peak would be 3:1 suggesting presence of some trimer present. (3)

(-2 for just aggregate)

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

(3) $LS \propto M \cdot c$; Measure polydispersity (M_w / M_n)

Light scattering measures a "weight average" M_w $\left(M_w = \frac{\sum n_i M_i^2}{\sum n_i M_i}\right)$. Because of the M_i^2 term, large aggregates contribute substantially to LS signal even at low [ ]. 4

9. CD:

A) What is the single most common advantage of taking a CD measurement? And, what special aspects are needed for the instrument to carry out CD measurements?

2 CD
1 Inst.

(3) CD measures the difference in absorbance between L- and R-circularly polarized light. CD will be non-zero when dealing with a chiral molec. CD at what λ sensitive to fold. Inst. needs special lamps and cuvette to work in UV ~ 200 nm.

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?

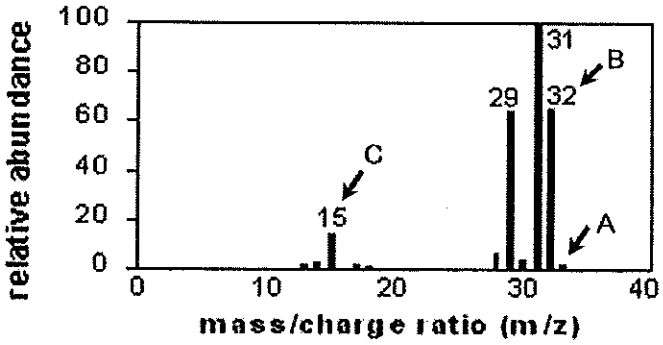
(3) A)  B)  } Molecules A & B are enantiomers of each other. Chiral

10. Mass Spec:

a) Name the three major / general components of a typical mass spec instrument.

(3) Ionizer (1) Analyzer (1) Detector (1)

b) Consider the following mass spec obtained from methanol (CH_3OH). Identify the ion peaks A, B & C (by isotope composition and charge) for the corresponding **cations** on the blanks provided.



(6)

$13+3+16+1=33$

A = $[^{13}C^{1}H_3{}^{16}O^{1}H]^+$ (2)

B = $[^{12}C^{1}H_3{}^{16}O^{1}H]^+$ (2)

C = $[^{12}C^{1}H_3]^+$ (2)

Must indicate non-std isotopes

b) Estimate the mass of a protein from the following mass spec data obtained from an ESI run. A set of sixteen peaks were obtained with peaks "n" and "n-2" having mass-to-charge ratios of 3872 and 4646. Estimated mass = 46,460 (show work for credit).

(5)

$M = n(3872) = (n-2)(4646)$

$3872n = 4646n - 9292$

$774n = 9292$ or $n = 12$ and $M = 12(3872)$

(2)

c) What do the acronyms **MALDI / TOF** stand for and what is the basis for measuring mass using this analysis technique?

(4) (1) MALDI = Matrix Assisted Laser Desorption Ionization

(1) TOF = Time of Flight

MALDI is used to make ions which are accelerated by voltage V,

(2) Taking on energy $E = V = \frac{1}{2}mv^2$ with velocity v, Then allowed to drift. TOF is used to calc. "v" $\rightarrow$ m/z

(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)