Fall '12 **CH370** 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); $s = v/\omega^2 r$
$\rho_{water} = 1.00$ g/cm^3; $s = M(1 - \nu'\rho)/N^0 f$; $(1/c_r)(dc_r/dr) = M\omega^2 r(1 - \nu'\rho)/RT$;
$A = A_0 \exp(-kt)$; $k = 1.38 \times 10^{-23}$ 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); $N^0 = 6.02 \times 10^{23}$ 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 trimeric protein has a molar extinction coefficient of 211,000 M^{-1}cm^{-1} at 280 nm. The molecular weight of the trimeric protein is 187,000 g/mol. A sample of the protein in a 0.20 cm cuvette was found to have a T = 0.50 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) $A = -\log T = -\log(.50) = \boxed{0.30}$ (3)

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

(2) $|E(1\%) \cdot MW| = |10 \cdot \varepsilon| \Rightarrow E(1\%) = \frac{10 \cdot \varepsilon}{MW} \left(\frac{g}{dL}\right)^{-1} cm^{-1} = \boxed{11.3 \left(\frac{g}{dL}\right)^{-1} cm^{-1}}$ (1) (11 cm^{-1}/g)

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

(3) $A = \varepsilon_M [M] \cdot l \Rightarrow [M] = \frac{A}{\varepsilon_M l} = \frac{0.30}{(211{,}000\ M^{-1}cm^{-1})(0.20\ cm)}$

$[M] = \boxed{7.11 \cdot 10^{-6} M}$ (7.11 μM) (1)

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

(3) $A = E^{1\%} \cdot c \cdot l \Rightarrow c = \frac{A}{E^{1\%} \cdot l} = \frac{0.30}{11.3 \left(\frac{g}{dL}\right)^{-1} cm^{-1} (0.20\ cm)} = 0.133 \frac{g}{dL}$

$c = 0.133 \frac{g}{dL} \times \frac{1\ dL}{100\ mL} \times \frac{1000\ mg}{g} = \boxed{1.33\ mg/mL}$

2. FRET: You have available Burnt Orange-31 and Raider Red-22 as a pair of chromophores that have a $R_o = 66.0$ Å (6.60 nm) for possible use in a FRET experiment on RNA folding that will require at least 20% efficiency. What is the maximum distance that you can expect to make meaningful measurements using these two chromophores? 83 Å (Show Work for credit.)

(6) (1) $\boxed{Eff = \frac{1}{1 + \left(\frac{R}{R_0}\right)^6}}$ or $Eff. = \frac{1}{1 + x^6}$ where $x = R/R_0$

$0.20 = \frac{1}{1 + x^6} \Rightarrow 0.20 + 0.20x^6 = 1$ or $x^6 = \frac{0.80}{0.20} = 4.0$

$\Rightarrow x = 1.26$

$\boxed{R = 1.26 R_0 = 83 Å}$

3. **Radioactivity:** Consider an isotope "X" with 74 protons and 101 neutrons.

(1) **A)** What is the element? W (1)

175
74W

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

(6) **B)** X → $^{175}_{75}Re$ (1) + beta⁻; X → $^{171}_{72}Hf$ (1) + alpha; X + EC → $^{175}_{73}Ta$ (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 **0.010 / yr.**

i) Calculate the **half-life** of the radioisotope. **Half-life =** 69.3 yr (4)

Show work here:

(4) $t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{0.010/yr} = 69.3\ yr$ (2)

ii) How many **years** will it take for 99% of a sample of this radioisotope to undergo radioactive decay ? 461 yr. **years.** Show work here:

(5) $A = A_0 e^{-kt} \Rightarrow 1 = 100\, e^{-kt}$ or $0.01 = e^{-0.010/yr \cdot t}$ (2)

$\rightarrow t = \frac{\ln(0.01)}{-0.01}\ yr = 461\ yr$

4. SDS-PAGE:

A) In SDS-PAGE, compared to the stacking gel, the running gel is usually:

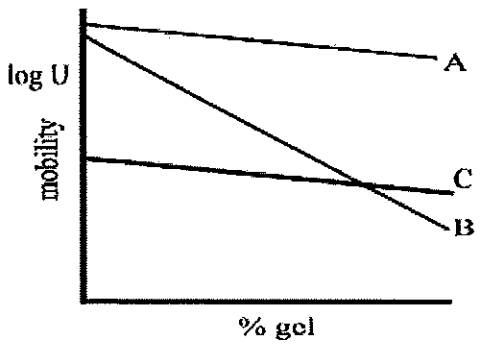
(2) **(a)** Higher pH, higher % acrylamide **b)** Lower pH, lower % acrylamide
c) Higher pH, lower % acrylamide **d)** Lower pH, higher % acrylamide

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

(3) Subunit composition (e.g. α2β2): $\alpha_2\beta$ (3).

≈ 1·60
≈ 2·30

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 smallest total charge under the conditions of these mobility experiments?



(4) Largest: B (2)

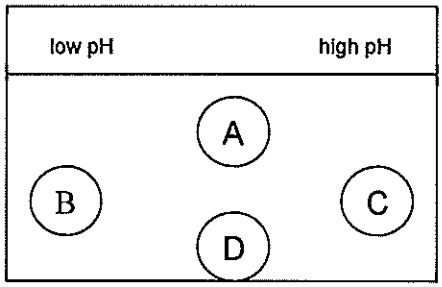
Least total charge: C (2)

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 (1); B = P2 (1); C = P3 (1); D = P4 (1).

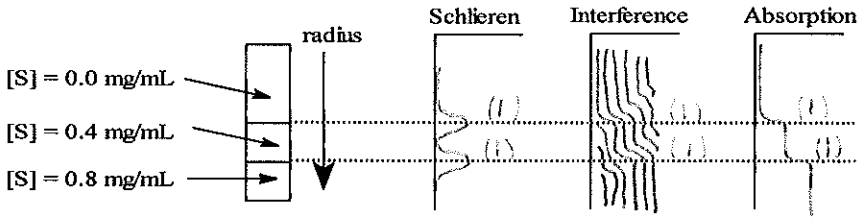
(4)

P1= FAGRRALVEDPIW
P2= AGWDPLEFD
P3= IKLRGAKPV
P4= DDGAKR



7. Optics: Consider the following low-speed, diffusion run looking at an ultracentrifuge sample cell with three liquids layered over each other as 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. Consider a properly folded, 200 kDa globular protein. Estimate both the **value and units** of "s" and "radius" (*within one order of magnitude*), and just the **units** of "D" expected for this protein.

$\frac{x}{20} = \left(\frac{200}{1000}\right)^{2/3}$

(6)

a) s ~ $7 \cdot 10^{-13}$ sec (1) (1) b) radius 10 Å (1) (1) c) D units cm^2/sec (2)

$Vol = \frac{M \cdot \bar{v}}{N^\circ} = \frac{4}{3}\pi R^3$

9. Analytical Ultracentrifugation:

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

(2) size (mass) (1) shape (1)

B) Sedimentation velocity: The sedimentation coefficient (s) of a protein is suspected to be around 10S. Calculate the rotor speed in rpm to use for a sedimentation velocity run if we want the boundary to move from position $r_1 = 6.75$ cm to $r_2 = 7.75$ cm over a time interval of 60 min.

(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)

(6)

$\ln\left(\frac{r_2}{r_1}\right) = s \cdot \omega^2 (t_2 - t_1)$;

$\rightarrow \boxed{\omega^2 = \frac{\ln(r_2/r_1)}{s \cdot (t_2 - t_1)}}$ (1)

$s = 10S = 10 \cdot 10^{-13}$ sec (2)

$r_1 = 6.75$ cm ; $r_2 = 7.75$ cm

$t_2 - t_1 = 60$ min $= 3600$ sec (1)

$\omega^2 = \frac{0.138}{(10 \cdot 10^{-13}\ sec)(3600\ sec)} = 3.83 \cdot 10^7\ sec^{-2}$ or $\omega = 6190$/sec (3)

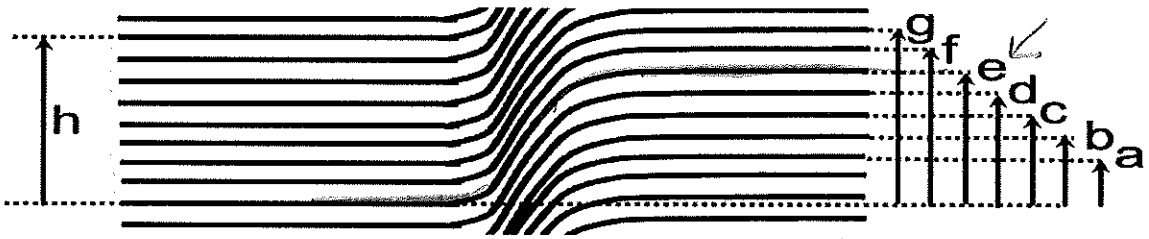
$\rightarrow rpm = \frac{6190}{sec} \times \frac{60\ sec}{min} \times \frac{1\ rev}{2\pi} = 5911$ rpm (6)

$\boxed{\omega = 59{,}100\ rpm}$ 3

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 = 6500 rpm, the radius to the sample optics section is 7.30 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 shown below are obtained where a = 3.92 mm, b = 5.59 mm, c = 7.27 mm, d = 8.97 mm, e =10.64 mm, f = 12.35 mm, g = 14.06 mm and h = 13.40 mm.

Calculate the concentration of the protein sample at the arrow (↓) in mg/mL from the data given above. Place answer here 1.55 mg/mL, show work below.

7 pts with work



(7) $\Delta J = |e|/(h/8) = \frac{10.64\text{ mm}}{(13.40\text{ mm}/8)} = 6.35$ (3) (1)

$$\Delta c \sim C = \frac{\Delta J \cdot \lambda}{a \cdot K} = \frac{(6.35)(546 \cdot 10^{-6}\text{ mm})}{(12.00\text{ mm})(0.186\ (\text{g/cm})^{-1})} = 0.00155\ \text{g/cm}^3$$

(2 pts)

$C \sim 1.55\ \text{mg/cm}^3$

D) You have sequenced the gene of your favorite protein, and you know it is composed of subunits containing 457 amino acid residues for a subunit molecular weight of 50,627 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 a decamer (10 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. To get the best data from the experiment, you would like to have 5 times the concentration at r_2 (7.50 cm) than at your reference point r_1 (7.00 cm). Assuming your protein to be a dodecamer, **calculate the rotor speed in rpm** that you should run your sedimentation equilibrium experiment to achieve the **5x difference in concentration between r_1 and r_2.**

(8) $T = 20°C = 293K$ (1); $M \approx 12 \cdot 50{,}627 = 607{,}524$ g/mol for dodec.

$r_2 = 7.50\text{cm}\ (r_2^2 = 56.25\text{cm}^2)$; $r_1 = 7.00\text{cm}\ (r_1^2 = 49.00\text{cm}^2)$

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

(2) Eqn.
(1) T in K
(1) $\bar{v}$
(2) r's
(1) right

$$\omega^2 = \frac{2 \cdot R \cdot T \cdot \ln c_2/c_1}{M(1-\bar{v}\rho)(r_2^2 - r_1^2)} = \frac{2(8.314 \cdot 10^7\ \text{g}\cdot\text{cm}^2/\text{sec}^2\cdot\text{mol}\cdot\text{K})(293\text{K})\ln(5)}{(607524\ \text{g/m})(1-0.735)(56.25-49.00\ \text{cm}^2)}$$

1.609; 0.265; 7.25 cm^2

$\omega^2 = 67161\ \text{sec}^{-2}$

$\omega = 259/\text{sec} \cdot \frac{60\text{ sec}}{\text{min}} \cdot \frac{1\text{ rev}}{2\pi} = \boxed{2473\text{ rpm}}$ (2)

8 pts with work

10. Light Scattering:

A) What is the relationship between the intensity of scattered light as a function of the wavelength (λ) and distance from source (r) (I is proportional to λ, or $1/\lambda$, etc., similarly with "r"):

(2) i) Wavelength: I is proportional to $1/\lambda^4$ (1); ii) Distance: I is proportional to $1/r^2$ (1)

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) Briefly describe in general terms what is measured when using "dynamic" light scattering, how the results are reported, and what advantages there are to using dynamic vs. static light scattering. **(3-4 sentences)**

(4) Dynamic LS measures scattering fluctuations over very short time intervals which can yield "D" - the diffusion (2) constant. $D \rightarrow f\left(\frac{kT}{D}\right)$ and $f = 6\pi\eta \cdot R_h$. Report results in hydrodynamic radius (1) instead of "D" or fluctuation. Advantage is R_h can be measured over larger range (1) than R_G (R_G 100-1000Å) (R_h 10-10,000Å)

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?

(4) ($LS \propto L^2 \cdot M$, $RI \propto L^2$) → A small amount of trimer will have 3X the relative LS signal compared to the monomer. Monomer (2:1) → Trimer (6:1)

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

(3) $LS \propto L^2 \cdot M$; Since the LS signal is proportional to $L^2 \cdot M$, the signal from a 1% contaminant of $M \sim 10^6$ would be as strong as 99% of the sample at 10K.

11. CD: Name two kinds of useful information that can be obtained from a CD spectrum?

(4) 2 pts each: Monitor folding; Predict secondary structure; Thermal stability; Binding

(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)