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BIOC 463A
October 4, 2001

First Hour Exam

Please put your name on each page. There are five pages, each page is worth 10 points for a total of 50 points. *This exam will represent 10% of your total grade for the course.*

Please answer all questions briefly.

For all calculations, be sure to include the proper units.

Consider it pure joy, my brothers, whenever you face trials of many kinds, because you know that the testing of your faith develops perseverance. Perseverance must finish its work so that you may be mature and complete, not lacking anything. (James 1:2-4)

Useful Equations:

$$A = ecl \quad \text{and} \quad \ddot{A}A = (\ddot{A}e)(\ddot{A}c)(l)$$

$$c^2 = a^2 + b^2$$

$$C_1V_1 = C_2V_2$$

$$\text{pH} = -\log [\text{H}^+]$$

$$\text{pH} = \text{pK}_a + \log [\text{CB}]/[\text{WA}]; \quad \text{pK}_a \text{ values: Phosphate (6.8); Tris (8.1).}$$

$$[\text{LR}] = \frac{[\text{R}_t][\text{L}]}{[\text{K}_d] + [\text{L}]} \quad \text{and} \quad \text{K}_d = \frac{[\text{L}][\text{R}]}{[\text{LR}]}$$

$$\text{IC}_{50} = \text{K}_{L2, \text{app}} = \text{K}_{L2} \left(1 + \frac{[\text{L1}]}{\text{K}_{L1}} \right) \quad \text{and} \quad \text{K}_{L2} = \frac{\text{IC}_{50}}{1 + \frac{[\text{L1}]}{\text{K}_{L1}}}$$

$$\text{K}_{\text{av}} = \frac{V_e - V_o}{V_t - V_o}$$

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1. (2 pts.) (a) Calculate the pH of 1L of a Tris buffer, prepared by mixing 50 mL of 1M TrisHCl with 50 mL of 1 M Tris base then adding 900 mL of H₂O.

The solution to this is quite simple if you realize that equal moles of TrisHCl and Tris base are being added, therefore, the pH = pKa = 8.1.

A second method is to calculate [TrisHCl] and [Tris]:

(50 ml)(1M TrisHCL)=(1000 ml)([TrisHCl]) so [TrisHCl] = 0.05 M = [Tris]

**and pH = pKa + log [Tris]/[TrisHCl] = 8.1 + log (0.05)/(0.05)
= 8.1 + 0 = 8.1**

(b) Calculate the total Tris concentration of this buffer.

$$[\text{Tris buffer}] = [\text{TrisHCl}] + [\text{Tris}] = 0.05 \text{ M} + 0.05 \text{ M} = 0.1 \text{ M}$$

2. (3 pts) (a) The ionic strength of a solution can not be measured directly. However, it is related to what property of a buffered solution.

As we measured in Expt. 1, the ionic strength of a buffer is related to the conductivity of the solution. This is the property, which you actually measure.

(b) Calculate (or give a close approximation) of the ionic strength of the above Tris buffer.

Based on the results from Expt. 1, and the approximation discussed in class, for a Tris buffer near its pKa, ionic strength = $\frac{1}{2}$ [Tris buffer] = 0.05 M = 50 mM.

3. (5 pts) The pH range in which a buffer is effective is given as pKa \pm 1. Fill in the Table below and briefly explain why this relationship, and the values in the table, adequately describes the effective pH range of a buffer.

<u>pH</u>	<u>[CB]/[WA]</u>
pKa -1	<u>1/10</u>
pKa	<u>1/1</u>
pKa +1	<u>10/1</u>

When pH < pKa -1, the [CB] is too low to effectively consume added acid, therefore the pH of the solution would change. When pH > pKa +1, the [WA] is too low to consume added base (i.e. OH⁻), and likewise, the pH would also change significantly.

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4. (2 pts) The milliMolar extinction coefficients at 500 nm for free and avidin-bound HABA are $0.6 \text{ mM}^{-1} \text{ cm}^{-1}$ and $34.5 \text{ mM}^{-1} \text{ cm}^{-1}$, respectively. Calculate the total absorbance at 500 nm for a solution containing 95 μM HABA and 5 μM HABA-avidin complex (cuvette pathlength = 1 cm).

$$\begin{aligned} A_{500} &= (0.6 \text{ mM}^{-1} \text{ cm}^{-1})([\text{HABA}])(1 \text{ cm}) + (34.5 \text{ mM}^{-1} \text{ cm}^{-1})([\text{HABA-avidin}])(1 \text{ cm}) \\ &= (0.6 \text{ mM}^{-1} \text{ cm}^{-1})(0.095 \text{ mM})(1 \text{ cm}) + (34.5 \text{ mM}^{-1} \text{ cm}^{-1})(0.005 \text{ mM})(1 \text{ cm}) \\ &= 0.229 \end{aligned}$$

5. (3 pts) List in decreasing order of their respective contribution, the three amino acids that contribute to the absorbance of a protein at 280 nm.

Tryptophan ($5,500 \text{ M}^{-1} \text{ cm}^{-1}$) > Tyrosine ($1,490 \text{ M}^{-1} \text{ cm}^{-1}$) > cystine ($125 \text{ M}^{-1} \text{ cm}^{-1}$)

6. (5 pts) Briefly, describe the general procedure employed to determine protein concentration using either the Bradford, Lowry, or BCA methods. The concentration of protein determined by this method is typically given in what units?

- (1) Dilutions of stock BSA are made to give range of [BSA] in mg/ml.***
- (2) Colorimetric assay is performed by adding appropriate amount of reagent to protein and allowing color change to occur.***
- (3) Absorbance at appropriate wavelength is measured, and standard curve of Absorbance vs. [BSA] is prepared.***
- (4) Unknown protein sample is prepared in the same manner. The absorbance of the unknown sample is then related to its concentration using the standard curve.***

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7. (2 pts) Inhibitor #1 has an $IC_{50} = 20 \mu M$, while Inhibitor #2 has an IC_{50} value of $5 \mu M$ for the same receptor. Which inhibitor has the lower K_d for the receptor?

From the equation on the first page: $IC_{50} = K_{L2, app} = K_{L2} (1 + \frac{[L1]}{K_{L1}})$. Therefore, IC_{50} is

directly proportional to K_{L2} , the K_d for the inhibitor. Thus, inhibitor #2, has the lower K_d .

8. (3 pts) If you had three biotin analogues with K_d (M) values of 10^{-12} , 10^{-8} , and 10^{-3} , respectively, which analog would you choose to demonstrate a competitive binding assay of the HABA-avidin complex to a biochemistry laboratory course and why. Be brief in your answer.

The analog with the a $K_d = 10^{-8} M$ would probably be the best one to use, since there would be a reasonable degree of competition between this analog and HABA ($K_d = 3 - 7 \mu M$). However, one might be able to use the analog with $K_d = 10^{-12} M$, but you would perhaps run into the same sensitivity problems as we did with biotin. The last analog has a K_d that is too high to be useful. It would require the addition of large amounts of competitor.

9. (5 pts) Using the equation given on page 1 relating $[LR]$ to $[L]$, $[R_t]$, and K_d , rearrange the equation to give a linear transform (i.e. a double reciprocal equation), and draw the plot that you would expect to obtain from a ligand binding experiment. Indicate on the graph the range of data points for which you can have the most confidence. Show your math and label the figure properly.

$$\frac{[LR]}{[K_d] + [L]} \rightarrow \frac{1}{[LR]} = \frac{K_d + [L]}{[R_t][L]} \rightarrow \frac{1}{[LR]} = \frac{K_d}{[R_t][L]} + \frac{1}{[R_t]}$$

Plot $1/[LR]$ vs. $1/[L]$ (see Fig. 10-1C in N&B text). The slope = $K_d/[R_t]$ and the y intercept is $1/[R_t]$. The region of greatest confidence is found at the mid to lower values of $1/[L]$. This data corresponds to $[L]$ values that are close to K_d .

10. (2 pts) (a) List one type of gradient that can be used to elute a protein off an ion exchange column.

Salt gradient or pH gradient.

(b) What kind of interaction between the protein and resin are you disrupting.

Electrostatic or ionic interactions are disrupted by either a shielding effect (salt gradient) or by changing the net charge on the protein (pH gradient).

11. (3 pts) The ability of a protein to bind to an ion exchange column depends upon the pH of the buffer and the pI of the protein. Using the relationship given below, fill in the table:

pH	Total Charge on protein	Binds to this type of ion exchange resin
pH < pI	<u>Z > 0 (positive)</u>	<u>Cation exchange resin such as CMC.</u>
pH = pI	<u>Z = 0 (neutral)</u>	<u>Neither</u>
pH > pI	<u>Z < 0 (negative)</u>	<u>Anion exchange resin such as DEAE.</u>

12. (5pts) Often, during a protein purification procedure, one must take into consideration the next step in the procedure when setting up the current step. Using your column chromatography experiment as an example, explain how the buffer conditions of the G75 column relate to the next chromatographic step.

The second step of the procedure, ion exchange chromatography on CMC required two buffer conditions for the protein eluting from the G75 column. First, the ionic strength had to be low and the pH had to be less than the pI for either of the proteins. In this case, we used a 10 mM phosphate buffer with pH 6.0. This ensured that both proteins would be efficiently bound to the CMC resin, from which they could be differentially eluted by using an increasing pH gradient.

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13. (2 pts) List two functions of SDS in a SDS-PAGE experiment.

First, SDS denatures the protein, exposing the entire polypeptide. Second, SDS coats the protein (1 SDS/2 amino acids) with negative charge (-2/sulfate head group). This gives the protein a charge proportional to its mass. This final point is crucial because it ensures that the charge to mass ratio is the same for each protein, allowing for separation of proteins by size alone (see problem 15).

14. (3 pts) In the diagram below, show how an 18 kDa monomeric protein and a heterodimeric protein with subunits of 20 kDa and 30 kDa would behave on a SDS and native PAGE gels.

SDS gel: would have one band corresponding to 18 kDa for the monomeric protein and two bands at 20 and 30 kDa for the dimeric protein.

Native gel: would have one band corresponding to 18 kDa for the monomeric protein and one band corresponding to 50 kDa for the dimeric protein.

15. (5pts) Protein fractionation by SDS-PAGE is most similar to what type of column chromatography method? Briefly justify your answer.

Fraction by SDS-PAGE is most similar to gel filtration chromatography. Denaturation of the protein with SDS results in a polypeptide that is coated with a negatively charged lipid (SDS). However, the amount of charge is proportional to the molecular mass (size) of the protein. Therefore, the proteins are separated based on molecular mass alone.