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Practice Quiz, Chapters 1-3; August 24, 2010

Answer all questions to the best of your ability. Be concise.

1. The phosphate buffer system is important for buffering intracellular pH. If the total cellular concentration of phosphate is 20 mM, and the pH is 7.4, what is the concentration of the predominant phosphate species HPO_4^{2-} and $H_2PO_4^-$?
[Hint: the pKa of $H_2PO_4^- \rightarrow HPO_4^{2-}$ is 7.2] (9 points)

------- *Answer*

pH = pKa + log [A-] / [HA] (1.5 point)

7.4 = 7.2 + log [A-] / [HA] (1.5 points)

0.2 = log [A-] / [HA] (2 points)

1.58 = [A-] / [HA] (2 point)

Therefore, the amount of A- = 1.58 / 1 + 1.58 = 61.2%

61.2% of 20 mM = 12.25 mM [A-] = HPO_4^{2-} (1 points)
38.8% of 20 mM = 7.75 mM [HA] = $H_2PO_4^-$ (1 points)

2. List the four types of noncovalent interactions that are responsible for the interactions within and between macromolecules in living organisms. List them in the order of the potentially strongest to the weakest interactions, and give the range of their energies in kJ/mol. (4 points)

-----*Answer*

Charge-charge interactions, ~40-200 kJ/mol

Hydrogen bonding, ~2-20 kJ/mol

Hydrophobic effects, ~ 3-10 kJ/mol

Van der Waals interactions, ~0.4 – 4 kJ/mol

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3. You are carrying out undergraduate research in a research lab. The graduate student working with you previously made up three separate test tubes each containing one of the following amino acids: tyrosine, isoleucine and cysteine. The amino acids were dissolved in solution at pH 7. The graduate student had labeled the tubes with a black marker, but you mistakenly dipped the tubes into a dry ice-ethanol bath to freeze them for lyophilization. This caused the writing to come off the tubes. You want to use these amino acids as standards for an amino acid composition analysis that you plan to run that evening. You do not know where the dry amino acid stocks are, and you have no prior experience with the composition analyzer, thus you do not know how the different standards should run on the machine. The graduate student is on his way to Atlanta to fly out to a scientific meeting. You are expected to have results when the student returns from the meeting. After a moment of panic, you realize with calm intellectual prowess, that you can figure out which amino acid is which by using a spectophotomer and a positively charged spin column. Explain how you will distinguish the different amino acids from each other and explain why the methods work. (4 points)

------ *Answer*

The next time you will pay more attention to the solvent system you are using. However, with the damage already done, you take the absorption spectrum of each amino acid at 280 nm and you find that the contents of only one of the tubes absorbs at 280 nm, this tube holds the tyrosine. (2 points)

Then you reason that if you take an aliquot of the two remaining tubes, raise the pH of the aliquots to ~9, and pass the contents over a positively charged column, the cysteine should bind to the column most strongly since the sulfhydryl group (SH) will ionize at pH values above 8.4 to form (S^{-}). Thus, if you pass the amino acids over the column in a solution with no salt, and with a solution with some salt, the amino acid which elutes first from the column will be the isoleucine, while the other amino acid will be the cysteine. (2 points)