BIOL/CHEM 3361 Biochemistry I Fall 2017
(and BIOL 6352)

PROBLEM SET 1

Due: Wednesday Sept. 13, 2017 at 5:00 pm in the collection box in the hall outside FO 3.606.
Problem sets MUST contain a COVER SHEET that includes your:

- **Name**
- **Student ID**
- **Section Number (001 for Marsh/Lee, 002 for D'Arcy/Yoo)**
- **Total number of pages submitted, including the cover sheet**

Please also write your name on EACH page.
NO late problem sets will be accepted.

There are 31 lettered subparts to the questions below. Each will be graded on a 5 point scale, and then the summed score will be converted to a percentage of the total points.

For full credit, **all steps** to the solutions of the following problems must be shown. You may work together on the problems, but you may not copy or plagiarize. Your answers must be written, must show your own math steps, and must be in your own words.

Round your answers as appropriate, but in no case to no more than three significant figures.

For problems 1 and 2, assume an activity coefficient of 1 for all substances and no effect of ionic strength. Eliminate terms in quadratic solutions for $[H^+]$ only if the weak acid is dissociated < 5%. Reported pKa values can vary depending on the conditions under which they were measured; therefore, in solving the following problems use the pKa values given with the problems.

1. a. What concentration of HCl will have a pH of 4.0? (For HCl, pKa < 0)
   b. What concentration of benzoic acid will have a pH of 4.0? (For benzoic acid, pKa=4.20)
   c. What is the pH of 20 mM phosphoric acid? (For H_3PO_4, $pKa_1 = 2.12$, $pKa_2 = 7.21$, $pKa_3 = 12.32$)
   d. If 10 ml of 1 M phosphoric add is added to 25 ml of 1 M Na_3PO_4, and the total volume brought to 100 ml with water, what will be the pH of the solution?

2. You are developing an assay for a deaminase that catalyzes the following reaction,

$$R\text{-}NH_2 + H_2O \rightarrow R\text{-}OH + NH_3$$

   To generate a measurable amount of product you include 0.5 μmol of the amine in a 100 μl reaction buffered by 25 mM HEPES-HCl, pH 7.25. For the primary amine, pKa = 7.25; for HEPES, $pKa_1 = 3$, $pKa_2 = 7.55$; for ammonium, pKa = 9.25.
   a. What fraction of the HEPES will be HEPES^o (i.e., the protonated N form; see G&G Fig. 2-17) at the beginning of the reaction?
   b. If the concentration of the HEPES buffer is doubled, what fraction of the HEPES N will be protonated? Justify your answer.
   c. If all the amine is deaminated, what will be the final pH of the solution?

3. A patient is suffering from acute loss of HCO_3^- ions in the blood plasma resulting in a blood pH of 7.1. Normally blood pH is 7.4, $[HCO_3^-]$ is 24 mM, and $[CO_2(d)]$ is 1.2 mM. Assume a constant $[CO_2(d)]$ level in answering the following questions: (For formulas and a discussion of blood pH, see p. 46 of your text)
   a. What fraction of the HCO_3^- ion has been lost compared to normal blood?

b. If $NaHCO_3$ is given to the patient to add 10 mM to the blood, what would be the final pH?

4. Creatine (Cr) is made in the liver and kidneys and then transported to the muscles, where it serves as a high energy storage compound via phosphorylation by creatine kinase. Two forms of this enzyme exist, mitochondrial and cytoplasmic. In mitochondria there is net synthesis of creatine phosphate (CrP). In the cytosol, the phosphate is transferred from CrP to ADP to supply ATP to power the muscles. Table 3.2 of your G&G text lists $\Delta G^{\circ\prime}$ for hydrolysis of creatine phosphate: -43.3 kJ/mol at 25°C.

   a. Calculate the Keq′ for this hydrolysis.
   b. What are $\Delta G^{\circ\prime}$ and Keq′ for the synthesis of creatine phosphate from creatine and Pi?
   c. In mouse muscle cells at 25°C, [Cr] is 10 mM, and [CrP] is 20 mM. What concentration of Pi would be needed to maintain this level of phosphorylation by reversal of the hydrolysis reaction? Is this concentration of Pi a reasonable expectation? Why?
   d. Coupling ATP hydrolysis ($\Delta G^{\circ\prime}$ -35.7 kJ/mol at 25°C) to an unfavorable reaction is often used to drive biochemical reactions. Write the chemical equations for the CrP reverse hydrolysis and ATP hydrolysis and add them together to give the coupled reaction. Then calculate $\Delta G^{\circ\prime}$ for the coupled reaction. Is this reaction favorable? Justify your answer.
   e. Considering $\Delta G'$ in the muscle cells rather than under 1M standard state conditions, what ratio of ATP/ADP would be needed inside mitochondria to make the coupled reaction for CrP synthesis produce the concentrations of Cr and CrP observed in mouse muscle? Is this ratio reasonable? Why?

5. HMG-CoA reductase, a critical enzyme in cholesterol synthesis is the target for statin inhibitors. You are characterizing a possible new inhibitor and want to determine its mode of binding to HMG-CoA reductase. Equilibrium dialysis measurements at 25°C, 30°C, and 37°C yielded dissociation constants (K_s) of 2.5 x 10^{-8} M, 1.5 x 10^{-8}, and 1.0 x 10^{-8} M, respectively, for the HMG-CoA reductase-inhibitor complex.
   a. Is the binding becoming better or worse with increasing temperature? Explain.
   b. Using Excel or similar program, tabulate the data, make a van't Hoff plot, and use it to determine $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ for the dissociation reaction.
   c. What are the corresponding $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ for complex formation?
   d. What is $\Delta G^{\circ\prime}$ for complex formation at 25° C?
   e. Is this inhibitor binding driven by entropy or enthalpy? Explain.
   f. What mode of binding (i.e., forces or interactions) would you predict for the inhibitor? What's your rationale?

6. The tetrapeptide Ac-Asn-Met-Cys-Lys has its N-terminus blocked by acetylation. Its C-terminal pK_a is 4.30. The side chain pKa's are 7.8 and 10.4.
   a. Write this peptide sequence using single letter abbreviations.
   b. Draw its predominant chemical structure at pH 8.0, including charges.
   c. What fraction of the peptide will exist in the predominant form at pH 8.0? (Discount any species that represent less than 1% of all species.)
   d. Calculate the pI of the peptide.
   e. If this tetrapeptide is oxidized to link pairs the molecules via disulfide bridges, what will be the pI of the product?
   f. Can a mixture of the oxidized and unoxidized peptides be separated on a DEAE-matrix at pH 6.5? If yes, which peptide will elute first? Explain why.

7. Assume you are given a mixture of proteins that you analyze by standard 2-D electrophoresis, with the following results for isoelectric points and apparent molecular weights: protein A (M_r 22,400; pI 8.21), protein B (M_r 15,850; pI 5.43), protein C (M_r 89,200; pI 5.26), and protein D (M_r 128,200; pI 7.76).
   a. Approximately how many amino acid residues are there in protein C, and about how many of these carry a charge?
   b. If a 1.0 mg/ml solution of protein C at pH 6 has an absorbance of 0.63 at 280 nm in a standard 1-cm width spectrophotometer cuvette, and it is known from standard amino acid that 2.0 % of its residues are tyrosine, how many residues of tryptophan are there per protein molecule? (For an estimate of molar absorptivity ε, in units of M^{-1} cm^{-1}, see G & G Fig. 4-10 and round to one significant figure.)
   c. After the 2-D separation, which protein will be in the upper left quadrant of the gel, given that the cathode-proximal end of the isoelectric focusing gel was on the right side of the 2-D gel? Explain why, citing the physical basis of each separation step as part of your answer.
   d. If you want to binds these proteins to a P-matrix ion exchanger ($pKa_1 = 3$, $pKa_2 = 6$) and then elute them successively, what pH would you choose for the buffer and in what order would they elude with an increasing salt gradient? Justify your answer.
   e. If the mixture is subjected to salting out with ammonium sulfate at pH 6, which protein will precipitate last? Explain why.