

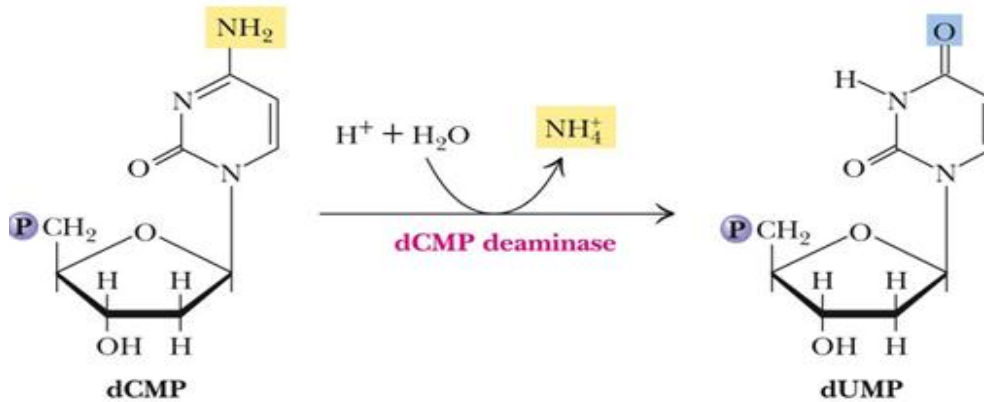
PROBLEM SET 1 (Q. 8a revised 9/7/12)

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 show your own math steps and be in your own words.

For problems 1-5, 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 pK_a values can vary depending on the conditions under which they were measured; therefore, in solving the following problems use the pK_a values given with the problems.

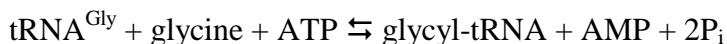
1.
 - a. What is the pH of 40 mM HCl?
 - b. What concentration of HCl will have a pH of 4.0?
 - c. If sufficient solid LiOH is added to the HCl solution in part b to titrate 90% of the HCl, how much will the pH change? (Discount any volume increase due to the addition of the solid LiOH.)
2.
 - a. What is the pH of 40 mM acetic acid ($pK_a=4.76$)?
 - b. What concentration of acetic acid will have a pH of 4.0?
 - c. If twice as much solid LiOH is added to the acetic acid solution in part b as was added to the HCl solution in question 1c, how much will the pH change? (Again, discount any volume increase due to the addition of the solid LiOH.)
 - d. Give 2 reasons why the pH change in question 2c is less than in 1c?
3.
 - a. The chelating agent EGTA (ethyleneglycol-O, O'-bis(2-aminoethyl)-N, N, N', N'-tetraacetic acid) is often used to selectively sequester Ca^{2+} in bioassays since it binds over 10^5 times stronger to Ca^{2+} than Mg^{2+} , the two major divalent cations in biosystems. If a solution of EGTA is made by dissolving 38 grams of the polyprotic acid in sufficient water to make a total volume of 1 liter. What is the pH of the EGTA solution? (For EGTA: $M_r = 380$, $pK_{a1} = 2.00$, $pK_{a2} = 2.65$, $pK_{a3} = 8.85$, $pK_{a4} = 9.46$)
 - b. How many ml of 1.00 M KOH and water must be added to the EGTA solution in part a to yield 2 liters with a pH of 7.8?
 - c. If you add an equal volume of 5.00 mM K_4EGTA to the EGTA solution in part b, what is the pH?
4. Normally blood is pH 7.4 and contains 24 mM HCO_3^- , 1.2 mM dissolved (d) CO_2 . Respiratory alkalosis, where blood pH rises due to a drop in dissolved CO_2 levels, is the most common acid-base abnormality observed in patients who are critically ill. It is associated with numerous illnesses and is a common finding in patients on ventilators.

- a. If respiratory alkalosis develops in a patient placed on a ventilator, with their blood pH elevated to 7.6, what fraction of the normal 1.2 mM $\text{CO}_2(\text{d})$ has been lost? Assume the $[\text{HCO}_3^-]$ drops by 2 mM for every 0.3 mM drop in $\text{CO}_2(\text{d})$. $\text{pK}_{\text{overall}}$ for the equilibrium between $\text{CO}_2(\text{d})$ and bicarbonate is 6.1 (see Blood Buffer in box on p. 45 of G&G text).
 - b. Usually over a 2-3 day period the kidneys begin to excrete enough HCO_3^- to bring the pH back to normal. Assuming the lowered level of $\text{CO}_2(\text{d})$ is unchanged, what will be the new level of $[\text{HCO}_3^-]$?
5. dCMP deaminase catalyzes the following reaction, as shown in Fig. 26.24a of the G&G text:



If 40 mM dCMP is completely deaminated by this enzyme in a reaction mixture containing 50 mM NaCl, 100 mM Tris·HCl (pH 7.1), what will the pH be at the end of the reaction? (For Tris, $\text{pK}_a = 8.1$) (For NH_4^+ , $\text{pK}_a = 9.24$)

6. For hydrolysis of ATP to ADP + P_i under standard temperature of 25°C , 0.25 M physiological ionic strength, and 3 mM Mg^{2+} , $\Delta G^{\circ'}$ is -32.5 kJ/mol and $\Delta S^{\circ'}$ is $5.37 \text{ J/}^\circ\text{mol}$.
 - a. What is $\Delta H^{\circ'}$ for this reaction?
 - b. Is this hydrolysis entropically or enthalpically driven? Explain.
 - c. What is the equilibrium constant for this reaction at 37° ?
 - d. What is the free energy change for this reaction in your resting muscles, where the ATP concentration is 10 times that of ADP, and P_i is 3 mM, as determined by P^{31} NMR spectroscopy?
7. In protein synthesis, tRNA^{Gly} is charged with glycine by the following reaction:



The $\Delta G^{\circ'}$ for hydrolysis of glycyl-tRNA is -33 kJ/mol . For other $\Delta G^{\circ'}$ values, use those given in Fig. 3.7 of the Mathews et al. Chapter 3 posted on the class eLearning site. Assume all values are for the standard temperature of 25°C .

- a. Calculate $\Delta G^{\circ'}$ for this charging reaction?
- b. What is the Keq' for this charging reaction?
- c. If glycine, ATP, AMP and P_i are present at typical intracellular values of 0.15 mM, 1 mM, 0.1 mM, and 3 mM, what will be the equilibrium ratio of charged to uncharged tRNA^{Gly} ?

- d. If glycyl-tRNA is consumed so that the ratio of charged to uncharged is decreased 10-fold and then 100-fold, what are the resultant $\Delta G'$ values for the reaction? For this calculation assume unchanging concentrations ATP, AMP, and P_i .
 - e. What effect does each 10-fold change in the ratio of charged to uncharged tRNA^{Gly} have on the $\Delta G'$?
8. The heptapeptide Lys-Ala-Gln-Met-Arg-Thr-Asp has pK_as of 4.5, 4.7, 9.5, 10.0, and 12.0. (You will need to assign the pK_as to particular groups.)
 - a. If this heptapeptide is ~~reacted with maleic anhydride and~~ cleaved by CNBr, what products will result?
 - b. Which hydrolysis product would arrive last at the detector of a TOF mass spectrometer using electrospray ionization from an acidic solution to volatilize and charge the peptides? Explain why.
 - c. If the hydrolysis products were chromatographed on CM-matrix at pH 7.0, which would likely emerge with the flow through during loading and which would stick to the column? Explain why.
 9.
 - a. Calculate the pI for the amino acid cysteine and draw its predominant chemical structure at its pI, including the + and - charges present. Use the pK_as for his given in Table 4.1 of the Garrett and Grisham text.
 - b. Calculate the pI for the tripeptide K-N-H and draw its predominant chemical structure(s) at its pI, including both essentially full (+ and -) and partial (δ^+ and δ^-) charges present. For partial charges, indicate what per cent of the molecules will carry the charge. Assume the N-terminal amino group has a pK_a of 9.5 and the C-terminal carboxylic group a pK_a of 4.3. For the amino acid side chains, use the pK_as given in Table 4.1 of the Garrett and Grisham text.
 10. Assume you are given a mixture of proteins A (M_r 18,245; pI 9.82), B (M_r 36,556; pI 7.44), and C (M_r 74, 172; pI 6.54) and are asked to separate them by SDS PAGE, isoelectric focusing, CM cation exchange chromatography, gel filtration, and salting out.
 - a. In the case of SDS PAGE, if proteins A and B migrate 12 cm while protein C migrates 8 cm, what can you conclude about protein B? Explain.
 - b. With isoelectric focusing, which protein will be closest to the cathode? Explain why.
 - c. With CM chromatography, what pH range should you use for the buffer and in what order will the proteins emerge from the column? Explain why.
 - d. In the case of gel filtration, in what order will the proteins emerge from the column? Explain.
 - e. With salting out, if you use pH 6.0 for the buffer, in what order should the proteins salt out? Explain.