Problem Set 1 (**Due Sepetmber 2nd**)

1. Look up the structures and pKa values for the buffers listed below (these are all commonly used in biochemical research). Justify the trend in pKa values you find based on the structures. (Note that it is a Nitrogen atom that accepts a, NOT the R-SO_3^- group)
    a. PIPES
    b. PIPPS
    c. HEPES
    d. MOPS
    e. MES

2. Generate a titration curve if 250 mM NaOH is titrated into 100 mL of 25 mM MOPS. Make sure to mark all of the important points on the X and Y axes.

3. Identify the primary role for each of the organelles in a eukaryotic cell:
    a. Nucleus
    b. Mitochondria
    c. Golgi apparatus
    d. Smooth ER
    e. Rough ER

4. O_2 is transferred from your lungs to muscle tissue by binding to hemoglobin; however, hemoglobin is not able to transport CO_2 back to the lungs.
    a. Write a balanced chemical reaction that describes how CO_2 is generated when O_2 reacts with glucose.
    b. The two products of this reaction can subsequently react together to form an acid.
        i. Write a balance chemical reaction for this process.
        ii. Determine the enzyme that catalyzes this reaction.
    c. Describe why these reactions are important in maintaining the pH of blood.

5. We'll learn this term the protein structure is not rigid. Proteins are very dynamic molecules that continuously sample multiple conformations including a fully unfolded form (denatured). Consider the simple equilibrium for the protein Ribonuclease A (RNase) that describes the folded and denatured states:

    RNase (denatured) $\rightleftharpoons$ RNase (folded)

    If the total protein concentration is 2 mM, using the data in the table below and thermodynamic expressions that you learned in General Chemistry (hint: think Van't Hoff Equation) answer the following question:

    a. Determine $\Delta H°$ and $\Delta S°$ for the folding reaction. Assume both values are temperature independent.
    b. Calculate $\Delta G°$ for RNase A folding at 25°C. Is this a spontaneous reaction?
    c. What is the denaturation temperature for RNase A under standard conditions?
    d. Standard autoclaves heat water to 121°C. Comment on why autoclaved water cannot be trusted to prevent RNase catalyzed RNA degradation.
    e. What temperature is necessary to make the ratio of denatured:foded RNase = 10?

Temperature (°C)	[RNaseA (denatured)]	[RNaseA (folded)]
50	5.1×10^{-6} M	2.0×10^{-3} M
100	2.8×10^{-4} M	1.7×10^{-3} M

6. Calculate the concentration of sodium acetate and acetic acid that are necessary to prepare a buffer solution of pH 5 that contains a total buffer concentration ([X] + [HX]) of 50 mM.

① MOPS $pK_a = 7.2$ MES $pK_a = 6.1$

I find the easiest way to compare these is by thinking about the Reactivity of the base. In class, we discussed this by justifying the Stability of the base. By extension, more stable base = less reactive base.

In the case of MOPS vs. MES the Electron withdrawing SO_3^- pulls e^- density away from the Nitrogen L.P. making it a weaker base; this is consistent with the pK_a (6.1 vs 7.2). This same argument works when comparing PIPES + PIPPS

PIPPS $pK_a = 7.96$ PIPES $pK_a = 6.76$

MES 6.1

PIPES and MES both have 2 carbons between the SO_3^- group + the Nitrogen base. MES, however, has an Oxygen in the ring which is more electronegative than the N, so it serves as an extra e^- withdrawing group & makes MES a weaker base than PIPES. This same trend holds true between MOPS + PIPPS.

HEPES 7.4

PIPES 6.76

HEPES is where things got less obvious. Examining the HEPES vs. PIPES structure, we see that HEPES replaces a SO_3^- with OH^-. If we assume that it's the $\sim N \sim OH$ that is the reactive base, then its easy to assume that HEPES is more basic b/c the OH is less e^- withdrawing than the SO_3^-.

② 100 mL 25 mM MOPS $pK_a = 7.2$

Beginning pH

	$HX + H_2O \rightleftharpoons$	X^-	$+ H_3O^+$
I	.025	0	0
C	$-x$	$+x$	$+x$
E	$0.025 - x$	x	x

$$10^{-7.2} = \frac{x^2}{.025 - x}$$

$$x^2 + 10^{-7.2}x - 10^{-7.2}(0.025)$$

1.577×10^{-9}

$$x = \frac{-10^{-7.2} + \sqrt{10^{-14.4} + 4(1.577 \times 10^{-9})}}{2} = 3.97 \times 10^{-5}\ M$$

$pH = -\log(\quad) = 4.4$

V @ eq pt. : $0.1\,L\left(\frac{25\ mmol}{L}\right) = 2.5\ mmol\left(\frac{L}{0.25\ mmol}\right) = 10\ mL$

V @ ½ eq. pt : $\frac{10}{2} = 5\ mL$

pH @ ½ eq pt = pK_a = 7.20

@ Eq Pt $V_{total} = 110$ mL so $[MOPS] = \frac{25\ mM\,(100\ mL)}{110\ mL} = 22.73\ mM$

$A^- + H_2O \rightleftharpoons HA + OH^-$

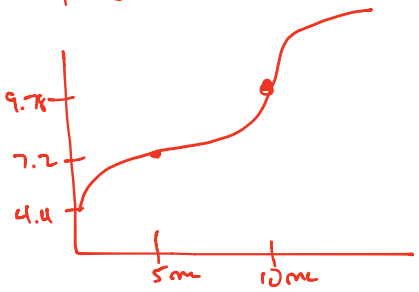
using ICE table, $[OH^-] = x = \frac{-10^{-6.8} + \sqrt{10^{-13.6} + 4(10^{-6.8})(0.02273)}}{2}$

$pK_b = 14 - 7.2 = 6.8$

$x = 5.99 \times 10^{-5}$

$pOH = 4.22$

$pH = 9.78$



(3) Nucleus: Contains and protects the genetic information

Mitochondria: location where many of the energy producing reaction occur

Golgi apparatus: Process proteins for secretion

Smooth ER: Fatty acid & cholesterol synthesis

Rough ER: Protein synthesis

(4) a) $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

b) i) $CO_2 + H_2O \rightleftharpoons H_2CO_3$

ii) carbonic anhydrase

c) The carbonic acid that is produced is in equilibrium with bicarbonate. Since both of these species are present any H^+ or OH^- that is produced by other chemical process will be consumed by reacting with H_2CO_3 or HCO_3^-.

$H_2CO_3 + H_2O \rightleftharpoons HCO_3^- + H_3O^+$

(5) a. $323.15\ K \rightarrow K = \frac{2 \times 10^{-3}}{5.1 \times 10^{-6}} = 392$

$373.15\ K \rightarrow K = \frac{1.7 \times 10^{-3}}{2.8 \times 10^{-4}} = 6.07$

$\ln \frac{K_2}{K_1} = \frac{\Delta H}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$ $\qquad \left(\ln \frac{392}{6.07}\right) R = \Delta H \left(\frac{1}{373.15} - \frac{1}{323.15}\right)$

$\Delta H = -83572\ J/mol$

pick either temp + calculate ΔG

$\Delta G = -8.314\,(373.15)\ln 6.07$

$\Delta G = -5595.41\ \frac{J}{mol} = \Delta H - T\Delta S$

$\frac{-5595.41\ J}{mol} = -83572 - 373.15\Delta S$

$\Delta S = -208.9\ J/mol\,K$

b. $\Delta G = -83572\ \frac{J}{mol} - 298.15\ K\left(-208.97\ \frac{J}{mol\,K}\right) = -21{,}268.1\ J/mol$

c. This is when the folding process becomes unfavorable ($\Delta G = 0$)

$0 = -83572\ \frac{J}{mol} - T\,(-208.97\ J/mol\,K)$

$T = 400\ K$

d. $121°C = 394.5\ K$ → this is below the denaturation temp of RNase A.

e. $K = \frac{1}{10}$ $\quad -RT\ln K = \Delta G = \Delta H - T\Delta S$

$T\Delta S - RT\ln K = \Delta H$

$T(\Delta S - R\ln K) = \Delta H$

$T = \frac{\Delta H}{\Delta S - R\ln K} = \frac{-83572}{-208.97 - 8.314\ln 1/10}$

$T = 440\ K$

(6) Acetic acid $pK_a = 4.75$

$50\,mM = X + HX$

$X = 50\,mM - HX$

$$pH = pK_a + \log \frac{X}{HX}$$

$$5 = 4.75 + \log \frac{50 - HX}{HX}$$

$$10^{0.25} = \frac{50 - HX}{HX}$$

$$1.778\,HX = 50 - HX$$

$$2.778\,HX = 50$$

$[HX] = 18\,mM$

$[X] = 50 - 18 = 32\,mM$