Problem C1
Modeling Acid-Base Equilibria
Due: April 4, 2006

In this assignment you will make a model of the net charge on an amino acid by considering the pK's of its ionizable groups and entering appropriate equations into a spreadsheet program. Consider the amino acid lysine, which has three ionizable groups: the carboxyl group (with a pK of 2.2), the alpha amino group (with a pK of 9.2), and the epsilon amino group (with a pK of 10.8). Starting from a very low pH (e.g., pH 1) at which the fully protonated form (H_3Lys^{++}) would be the main species, we can identify the major species produced as each group loses a proton: first the carboxyl (yielding H_2Lys^{+}), then the alpha amino group (yielding $HLys$), and finally the epsilon amino group (yielding Lys^{-}). For any ionizable group, the ratio of the base and acid forms at any pH can be found using the Henderson-Hasselbalch equation:

$$[B^-]/[HB] = 10^{(pH-pK)}$$

If only the four species listed above are considered to be present (but see extra credit question 10 below!), the ratios of their concentrations can be found using this equation:

$$\left(\frac{[H_2Lys^+]}{[H_3Lys^{++}]}\right) = 10^{(pH-pK_1)}$$

$$\left(\frac{[HLys]}{[H_2Lys^+]}\right) = 10^{(pH-pK_2)}$$

$$\left(\frac{[Lys^-]}{[HLys]}\right) = 10^{(pH-pK_3)}$$

The sum of the concentrations of these four species is the total concentraion of lysine.

$$Lys_{Tot} = [H_3Lys^{++}] + [H_2Lys^+] + [HLys] + [Lys^-]$$

This can be converted so that only [H_3Lys^{++}] and the ratios above remain by first dividing through by [H_3Lys^{++}] and expanding each ratio

$$Lys_{Tot} = [H_3Lys^{++}]\left\{1 + \frac{[H_2Lys^+]}{[H_3Lys^{++}]} + \frac{[HLys]}{[H_3Lys^{++}]} + \frac{[Lys^-]}{[H_3Lys^{++}]}\right\}$$

$$Lys_{Tot} = [H_3Lys^{++}]\left\{\begin{array}{l} 1 + \left(\frac{[H_2Lys^+]}{[H_3Lys^{++}]}\right) + \left(\frac{[HLys]}{[H_2Lys^+]}\right)\left(\frac{[H_2Lys^+]}{[H_3Lys^{++}]}\right) \\ + \left(\frac{[Lys^-]}{[HLys]}\right)\left(\frac{[HLys]}{[H_2Lys^+]}\right)\left(\frac{[H_2Lys^+]}{[H_3Lys^{++}]}\right) \end{array}\right\}$$

Once [H_3Lys^{++}] is found by solving this equation, the concentrations of the other species can be found in order by using the Henderson-Hasselbach ratios.
By the method outlined above, we can find the fraction of the total lysine present that is in each charged form, and also determine the total charge of all the species. The spreadsheet below shows one method for doing this.

	A	B	C	D	E
1	Ionization of Lysine				
2					
3	pH=	7			
4					
5	pK1=	2.2	Carboxyl	[H2Lys]/[H3Lys]=	=10^(B3-B5)
6	pK2=	9.2	Alpha amino	[HLys]/[H2Lys]=	=10^(B3-B6)
7	pK3=	10.8	Epsilon amino	[Lys]/[HLys]=	=10^(B3-B7)
8					
9	Ionic Form	Charge on form	Mole Fraction of form	Contribution to total charge	
10	H3Lys	2	=1/(1+E5+E6*E5+E7*E6*E5)	=B10*C10	
11	H2Lys	1	=C10*E5	=B11*C11	
12	HLys	0	=E6*C11	=B12*C12	
13	Lys	-1	=E7*C12	=B13*C13	
14					
15			Total Charge	=SUM(D10:D13)	

Questions (Total of 40 points)

1. Construct the model shown above. What is the major ionic form of lysine at pH 7? **Hand in written answer.**
2. The isoelectric point (pI) of a molecule is defined as that pH at which the sum of the charge on all ionic forms is zero. Use Goal Seek to find the isoelectric point of lysine (to the nearest tenth of a pH unit) by varying the pH in the model (cell B3) until the Total Charge reaches zero. Important: start your goal seek from a pH value of 7. **Hand in written answer.**
3. What is the major ionic form at this pH? **Hand in written answer.**
4. Modify the model so that it applies to tyrosine, for which pK1 is 2.2, pK2 is 9.1, and pK3 is 10.9 (the ionic forms are H_3Tyr^+, H_2Tyr, $HTyr^-$, and Tyr^{--}). Use a pH value of 7. **Submit a copy of the spreadsheet file (see below).**
5. What is the major ionic form at this pH? **Hand in written answer.**
6. Use Goal Seek to find the pI of tyrosine to 4 decimal places. Important: start your goal seek from a pH value of 7. **Hand in written answer.**
7. Reorganize the model for tyrosine so that it can calculate values of the total charge for more than one pH value (hint: put all calculations on a single row and then copy that row to create new rows for other pH values). Create one entry for every integer pH value from 1 through 14. Generate a plot of total charge versus pH. **Submit a copy of the spreadsheet file .**
8. Add Names for the pKs and pH values to your spreadsheet. **Submit a copy of the spreadsheet file.**

Extra credit

9. (10 points) Modify your spreadsheet to consider all eight possible ionic forms of lysine. **Submit a copy of the spreadsheet file.**
10. (10 points) (You must do question 10 first.) Generate a 3D surface plot of Total Charge as a function of pK_3 and pH using your spreadsheet from question 10. Let both pH and pK3 vary from 2 to 12 in increments of 2. **Submit a copy of the spreadsheet file.**

Submitting files via Blackboard

- Name your spreadsheet files according to the following format: **"lastnameC1Q#.???"** where **#** is the question number and **???** shows the file type (e.g., **.xls** for **Excel). An example would be MurphyC1Q8.xls.**

- **You can also submit the written answers on paper or in a Word document. If you use a Word document, name this file with your last name followed by "C1.doc" (e.g., MurphyC1.doc). You still need to electronically submit the spreadsheet files separately.**