1.) In lab, you're having fun with buffers based on a 0.1 M solution of sodium phosphate at pH 7.0. In this system, there are three pK_As: 12.7, 7.21, and 2.12.

A. (10 points) You may recall that in the pre-lab lecture on the first day, you were shown a chart indicating the relative amounts of different forms of phosphate (H_3PO_4, $H_2PO_4^-$, HPO_4^{-2}, PO_4^{-3}) needed to produce buffers of different pH. For your buffer conditions, please indicate to me the predominant phosphate species present, and the relative amounts of each at equilibrium.

$[H_2PO_4^-] = 0.0619$ M; $\%H_2PO_4^- = 62\%$
$[HPO_4^{-2}] = 0.0381$ M, $\%HPO_4^{2-} = 38\%$

B. (3 points) What is the concentration of hydroxide ion in this buffer?

pOH = 14-pH = 14 -7 = 7
$[OH] = 1.0 \times 10^{-7}$ M

C. (10 points) If you were to make the same buffer using the method of question #6 in chapter three, namely, starting with 1 M phosphoric acid and adding (in our case) sodium hydroxide until the pH reached 7.0, how many moles of sodium hydroxide would it take?

A.) Put 100 mL of 1.0 M H_3PO_4 in water to 800 mL or so (we will fill it up to 1000 mL after setting the pH). This gives 0.1 mol total H_3PO_4 and total phosphate.
B.) Add 0.1 mol HaOH to convert all H_3PO_4 to $H_2PO_4^-$
C.) Add 0.038 mol NaOH to convert 0.038 mol $H_2PO_4^-$ to HPO_4^{2-} to give amounts in part A.
D.) That is a total of 0.138 mol NaOH.

D. (7 points) If you used this buffer to carry out a separation of amino acids on the Dowex (cation-exchange; that is, negatively-charged beads) chromatography column that you are using this week in lab, what order would you predict the amino acids Histidine, Lysine, Glutamine, and Alanine to elute in?

A cation-exchange column retains positively-charged species, and the more highly-charged species should be retained longest. At pH 7, both glutamine and alanine should be essentially uncharged, they will elute first (in the wash), together. At pH 7, histidine will have a partial positive charge; it will be weakly retained and will elute well before lysine, which should be strongly charged at this pH.

2.) Imagine that lysine is dissolved in a buffer to a final concentration of 1 mM. In this buffer, 80% of the lysine's side-chain amines are protonated.

a) What would be the pH of this buffer? (15 points)

pH = 10.4

b) Please describe how you could construct 500 mL of a 1.0 M buffer at this pH using your choice of the following reagents:

fully-protonated glycine (the "free acid" form), MM = 76 g/mol, in dry solid
sodium salt of fully-deprotonated glycine, MM = 97 g/mol, in dry solid
HCl, MM = 36.5 g/mol, in solution at 14.5 M
NaOH, MM = 40 g/mol, in dry solid
water

Please provide masses and volumes of each reagent you choose, as appropriate. (15 points)

[HGly] = 0.286 M
[Gly^-] = 0.714 M

3.) Last year, one of my research students came across an experimental protocol recommended by a biochemical reagent supplier that involved a Tris buffer at pH 6.0 ("Tris" is a nickname for Tris hydroxymethyl amino methane). Please consult the following pK_A table and answer the questions below it.

Compound	**pK**
Oxalic acid	1.27
H_3PO_4	2.15
Citric acid	3.13
Formic acid	3.75
Succinic acid	4.21
Acetic acid	4.76
$Succinate^{-1}$	5.64
MES	6.09
Carbonic acid	6.35
PIPES	6.76
MOPS	7.15
HEPES	7.47
Tris	8.08
Bicine	8.26
Boric acid	9.24
$Bicarbonate^{-1}$	10.33
Piperidine	11.12
HPO_4^{-2}	12.38

a) Is Tris a compound you would choose for a buffer at pH 6.0? Please carefully explain to me why it is or is not. If it is not a chemistry you'd choose for this buffer, please suggest an alternative chemistry and justify your choice. (5 points)

Tris is not ideal for a buffer at this pH, as its pKa is more than 2 pH units away from 6.0. Thus, at pH 6.0, Tris would be >90% protonated, and would be a lousy buffer against added acid. MES and carbonic acid both have pKas near pH 6.0 and would thus be better choices.

b) What will be the concentration of hydroxide ions in this buffer? (3 points)

pH 6.0 = pOH 8.0
$[OH^-] = 1.0\times10^{-8}$ M

c) Given the compound you selected in part (a) for this buffer, please tell me the equilibrium concentrations you'd expect for the conjugate acid and conjugate base species in the buffer at pH 6.0. Assume that you are dealing with 1.00 liters of buffer at a total buffer concentration of 10.0 mM. (8 points)

- **Using carbonic acid, H_2CO_3:**
  $[A^-] + [HA] = 0.010\ M$
  $1.44668[HA] = 0.010\ M$
  $[HA] = [H_2CO_3] = 6.9\times10^{-3}\ M$ and $[A^-] = [HCO_3^-] = 3.1\times10^{-3}\ M$
- **For MES:**
  $[A^-] + [HA] = 0.010\ M$
  $1.8128[HA] = 0.010$
  $[HA] = [HMES] = 5.5\ mM$
  $[A^-] = [MES^-] = 4.5\ mM$

d) Suppose I want to make this buffer by dissolving in water some amount of the compound that you selected from the table, and then adding either strong acid (e.g., 6.0 M HCl) or strong base (e.g., 5.0 M NaOH) to get to the correct pH. Please tell me how many moles of your chosen compound I should start with, and how many milliliters of strong acid or strong base you predict I would need to add. (If you are unsure of your answer to part (c), assume here that the conjugate acid and base concentrations are each equal to 5.0 mM)
(5 points)

- **For carbonic acid, start with 10.0 mmol of carbonic acid, then add 3.1 mmol (= 0.62 mL) of 5.0 M NaOH to convert some of the acid to its conjugate base. Add water to 1.0 L, and it should be @ pH 6.0.**
- **For MES, again start with 10.0 mmol of the acid (call it "HMES"). Add 4.5 mmol (= 0.90 mL) of 5.0 M NaOH, and bring volume to 1.0 L.**

4.) Formic acid is a weak acid with a pK_a of 3.75. Its name comes from the Latin word "formica" ("ant") because it is very plentiful in the secretions of some species of ants, and is one of the irritants delivered by many stinging ants, bees and wasps.

a) Suppose that I were interested in using formic acid as the basis for a buffer for biochemical investigation. Would it be most useful for creating a buffered solution at pH 2, 4, or 6? Please briefly explain your answer. (5 points)

pH 4 – This is near the pKa. Thus, there will be adequate amounts of both conjugate acid and conjugate base to serve as an effective buffer.

b) If I use formic acid to create 2.00 liters of 50.0 mM buffer at the pH you selected in part (a), please tell me the equilibrium concentrations you'd expect for formic acid and its conjugate base. (10 points)

[Hform] = 0.017999 *M* → 0.0180 *M* formic acid
[form$^-$] = 0.032001 *M* → 0.0320 *M* formate

c) Suppose I want to make the buffer you described in part (b) by adding either strong acid (6.0 M HCl) or strong base (5.0 M NaOH) to a solution of concentrated formic acid in order to get to the correct pH. I know that I can buy formic acid from Sigma Chemical Corp. as an 88% solution (this works out to about 19 M). Please tell me

- what volume of 19 M formic acid I should start with
- whether I should add HCl or NaOH to get to the target pH
- how many milliliters of strong acid or strong base you predict I would need to add

(If you are unsure of your answer to part (b), assume here that the conjugate acid and base concentrations are each equal to 25.0 mM) (10 points)

- **How much stock formic acid?**
  2.0 L × (50 mmol/L) = 100 mmol total formate needed
  100 mmol = 0.100 mol
  0.100 mol × (1 L stock/19 mol) = 0.00526 L stock acid needed
  = 5.3 mL stock formic acid

- **HCl or NaOH?**
  Target pH involves getting a mixture of formic acid and formate anion. Thus, the starting formic acid must be partially deprotonated.
  This requires use of a base – NaOH.

- **Volume of NaOH needed?**
  Hform ↔ H^+ + form$^-$
  Start: **100 mmol/2 L**
  Target: **36 mmol/2 L** **64 mmol/2 L**

  So, I must add 64 mmol of NaOH to convert 64 mmol of Hform to form$^-$.
  64 mmol × (1 L/5000 mmol) = 0.0128 L = 13 mL of 5*M* NaOH

5.) In order to further explore the biochemistry of MSG action, we'd need to have an aqueous buffer in which to work. For this question, please assume that you need to make an 80 mM buffer whose pH is equal to the pK_a value that you gave for your answer to question 1(d). [If you have not answered 1(d), then use the default pH of 10 for this question.]

a) Please choose one of the following compounds that would be a reasonable choice for your buffer, and briefly explain the logic you used in making your selection. (Assume that you have access to all of these compounds in your stockroom; that is to say, the fact that you've never heard of them before should not bias you against any of these compounds.)
(5 points)

Compound	pK	
Oxalic acid	1.27	
H_3PO_4	2.15	
Citric acid	**3.13**	**Any of the highlighted species would**
Formic acid	**3.75**	**work just fine, as their pKa values are**
Succinic acid	**4.21**	**within one pH unit of the target pH of**
Oxalate^{-1}	**4.27**	**4.**
Acetic acid	**4.76**	
Succinate^{-1}	5.64	
MES	6.09	
Carbonic acid	6.35	
PIPES	6.76	
$H_2PO_4^-$	6.82	
MOPS	7.15	
HEPES	7.47	
Tricine	8.05	
Tris	8.08	
Glycylglycine	8.25	
Bicine	8.26	
Boric acid	**9.24**	**… and either of these would work for**
Bicarbonate^{-1}	**10.33**	**pH 10.**
Piperidine	11.12	
HPO_4^{-2}	12.38	

(data from Dawson et al (1986), *Data for Biochemical Research* (3rd ed), pp. 424-425)

b) Please tell me what you would expect the concentrations of the dominant buffer species to be at equilibrium. Please show all work for which you would like to receive credit. (10 points)

There are two key relationships here:

1.) pH = pKa + log [conjugate base]/[conjugate acid] and

2.) [conjugate base] + [conjugate acid] = 0.080 M

For a succinic acid buffer (pKa 4.21) at pH 4.0:

- **pH = 4.0 = pKa + log [conjugate base]/[conjugate acid] = 4.21 + log [Succ$^-$]/[HSucc]. Thus log [Succ$^-$]/[HSucc] = -0.21, and [Succ$^-$]/[HSucc] = 0.6166. This means [Succ$^-$] = 0.6166 [HSucc].**

- **Our second equation is [conjugate base] + [conjugate acid] = 0.080 M. So [Succ$^-$] + [HSucc] = 0.080 M. Substituting in, 0.6166 [HSucc] + [HSucc] = 0.08. So 1.6166 [HSucc] = 0.080, and [HSucc] = 0.04949 M.**

This means that [Succ$^-$] = 0.080 - 0.04949 = 0.03051 M

Does that make sense? Sure: the pH is slightly less than the pKa, so there should be a bit more of the conjugate acid than the conjugate base. Cool.

Final answer, then, is that at equilibrium, [HSucc] = 0.049 M and [Succ-] = 0.031 M

c) In lab, you have been making your phosphate buffer by making equimolar solutions of the relevant weak acid and conjugate base, and then mixing them until you reach the target pH of 7.0. I can think of at least three other ways to make a buffer solution. Please imagine that you are in a lab and have access to

- strong acid (6 M HCl)
- strong base (5 M NaOH),
- both the conjugate acid and conjugate base of the buffer species you have chosen, in powder form
- a pH meter
- a top-loading balance
- water, volumetric glassware, and other common lab resources.

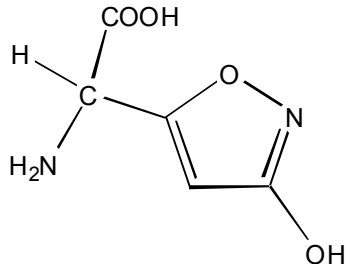
Please describe for me a way to make the buffer that you wrote about in parts (a) and (b). You need not provide quantitative measures; simple directions of the steps you'd need to follow will suffice. (5 points)

There are at least 4 ways to do this (any of these is an acceptable answer):

1.) Weigh out enough solid succinic acid and sodium succinate to reach the desired concentration, dissolve them in ~80% of the target volume, and then adjust the volume to the final amount after verifying the pH.
2.) Do what you've done in lab -- make up 80 mM solutions of succinic acid and sodium succinate, and then mix them until the target pH is reached.
3.) Start with 80 mmol of succinic acid dissolved in 800 mL of water for every liter final volume, adjust the pH to 4.0 with strong base, then bring the volume up to the final target.
4.) Start with 80 mmol of sodium succinate dissolved in 800 mL of water for every liter final volume, adjust the pH to 4.0 with strong acid, then bring the volume up to the final target.

Amino Acids

1.) The following compound is derived from the fungus *Amanita muscaria*. Like many fungal natural products, it has been suggested to have both toxic and hallucinogenic properties in humans.



A. (2 points) What type of molecule is it? (e.g., fatty acid, nucleotide, amino acid, sugar, acetal, phospholipid)

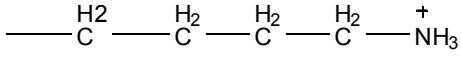
Amino acid

B. (3 points) This molecule contains a number of hydrogen bond donors and acceptors. What would you predict its net molecular charge to be at pH 6?

- Protonated N-terminus: $^{+}NH_3\rightarrow$ pKa ≈ 10
- De-protonated C-terminus: $\leftarrow COO^{-}$ pKa ≈ 2
Unlikely to protonated side chain: (+) + (-) = 0 net charge

2.) (5 points each) For each of the following, please give me: its name, its structure, its one-letter abbreviation, all relevant pK values, and the net charge you predict it to have at pH 8.5. Note that there is not necessarily only one correct answer to each of these -- pick one you like!

A. An amino acid which contains an epsilon-amino group.

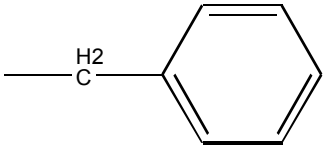
Lysine (K) **R-group:**  **Net Charge of +1**

pK ≈ 11

Note: all have pK_{NH_3} ≈ 10, pK_{COOH} ≈ 2

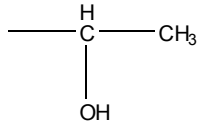
B. An amino acid which contains an aromatic side-chain.

Phenylalanine (F): no side chain pK (charge ≈ 0)

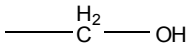
R = 

3 C. An amino acid which contains an alcohol group.

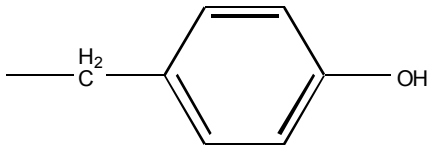
Threonine (T): no (common) side chain pK (charge ≈ 0)

R = 

Serine (S): no (common) side chain pK (charge ≈ 0)

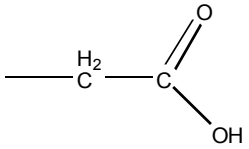
R = 

Tyrosine (Y): no (common) side chain pK (charge ≈ 0)

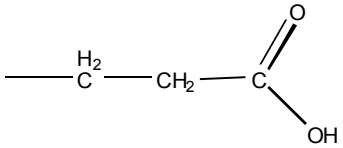
R = 

3 D. An amino acid which contains an acidic side-chain.

Aspartate (D): $pK_R \approx 3.9$ (4 is close enough), charge ≈ -1

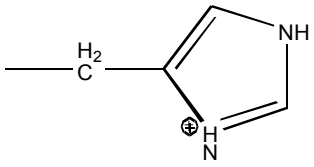
R = 

Glutamate (E): $pK_R \approx 4$, charge ≈ -1

R = 

3 E. An amino acid which has a pK_A for one of its ionizable groups that is near human physiological pH.

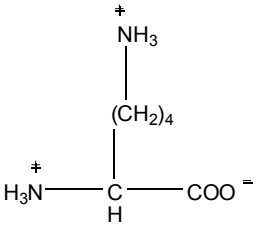
Histidine (H): $pK_R \approx 6$, charge at pH 8.5 ≈ 0

R = 

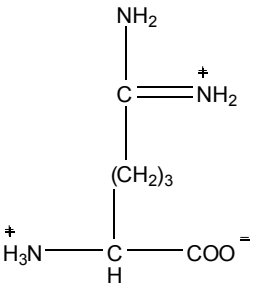
3.) Imagine that equimolar amounts of lysine, arginine, histidine, and asparagine are dissolved in 200 mL of the buffer that you have described in #1b. [If you were having difficulties with that question, you may simply declare an arbitrary pH at which to imagine this solution. If you choose this approach, be sure to tell me what pH you are using!] Please justify your answers. (4 points)

a) Please draw the structures of these amino acids, indicating for each the name, the one-letter code, and the approximate charges at this pH for each ionizable group. (12 points)

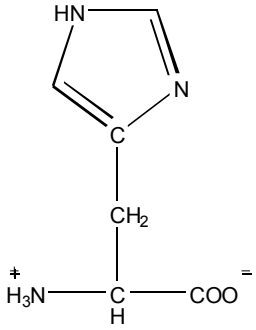
* **Lysine (K):



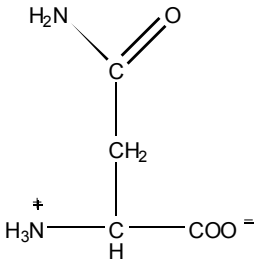
**Arginine (R):



**Histidine (H):



**Asparagine (N):



*** side chain NH_3 will be mostly protonated**
**** at pH=10.4, the alpha NH_3 will be more than 50% deprotonated, and so will carry a charge of between +1/2 and 0**

b) If you were to take this mixture of amino acids, and add some of the same Dowex cation-exchange resin that you used last week in lab, what would be the relative attraction of each amino acid to the resin? (e.g., which would be most strongly associated with the resin? which the least?) (8 points)

Dowex is a cation-exchange and therefore binds cations. Thus, the more strongly positive amino acids will be more strongly attracted to it. At this pH, asparagine and histidine will have a net negative charge and won't bind at all; lysine will be very weakly positive; and arginine will still be definitely positively.

c) in eluting your crude cytochrome *c* from your Dowex resin, you were able to monitor for presence of cytochrome *c* by visible pink color and absorbance of light at 410 nm. To detect the amino acids in part (b) as you elute them from the resin, it would be nice to have a similar monitoring scheme.

i. would you expect any of these amino acids to absorb light of about 280 nm?
ii. F-DNB reacts with primary amines. Would you expect it to react with any of these amino acids?

i.) none of these AA will absorb @ ~280 nm since none are aromatic
ii.) all of these AA contain a primary amine – the alpha amine – and so all should react with F-DNB

4.) Digestion of proteins in the stomach is accomplished with the aid of the enzyme pepsin. The activity of this enzyme requires the deprotonation of a pair of aspartic acid residues in the active site.

a) This poses a problem at the normal stomach pH of ~2. Why? What is the expected charge on the side chain of aspartic acid at this pH? (An approximate value is fine here, backed up by a *brief* explanation.) (7 points)

Normally, we would expect Asp to be fully (99%) protonated as this is 2 pH units below the pKa. This gives an expected charge of ~0. If Asp needs to be deprotonated, then this is a problem!

b) What is the [OH-] at this pH? (3 points)

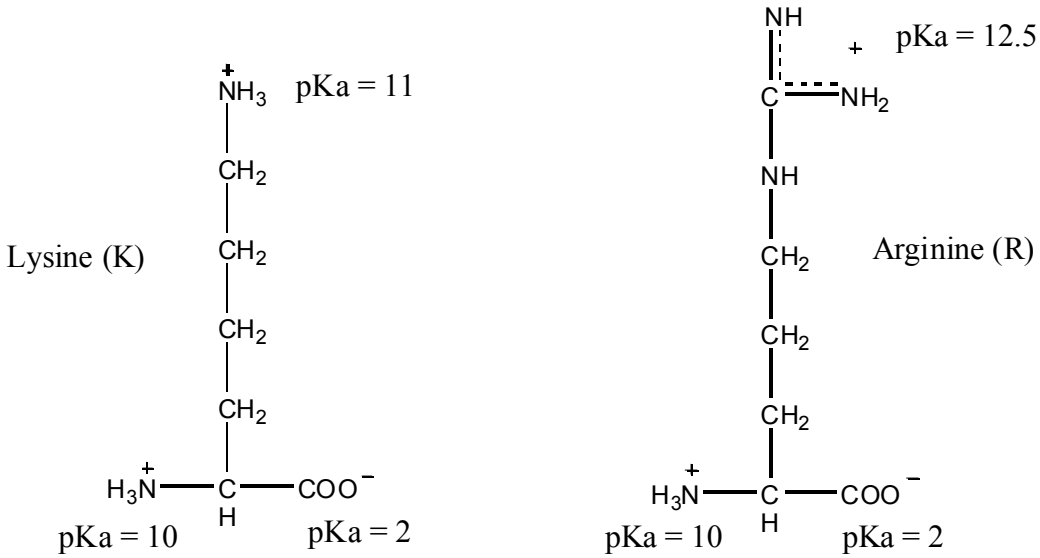
$[OH^-] = 10^{-12}$ *M*

c) To study this interesting biochemical puzzle, you could purify large quantities of pepsin and test it carefully under controlled conditions. To approximate the enzyme's normal biochemical environment, you'd probably want to perform your experiments in a buffer at pH 2. Pick an appropriate buffer system from the list displayed by the overhead projector, and determine how many moles of the conjugate acid and how many moles of the conjugate base you'd need to make 250 mL of a 300 mM buffer solution. (20 points)

- **Oxalic acid (pKa = 1.27) and phosphoric acid (pKa = 2.15) would both work, but clearly phosphoric acid is better since its pKa is closer to our target pH. Therefore, it should serve as a good buffer against both acid and base addition.**
- **If $[H_2PO_4^-] + [H_3PO_4] = 300$ *mM*:**
  $1.7079\ [H_3PO_4] = 300$ *mM*
  $[H_3PO_4] = 175.6$ *mM*
  $[H_2PO_4^-] = 124.3$ *mM*
  Therefore, H_3PO_4 in 250 mL = 44 mmoles and $H_2PO_4^-$ in 250 mL = 31 mmoles.

5a.) Please draw the structure of **one amino acid that matches each of the following** descriptions. Label each with its name and one-letter code, and indicate the pK_a values of ***all*** commonly-ionized groups. These ionizable groups should bear a charge appropriate to the indicated pH. You may wish to read question 4b before making your choices of which amino acids to draw here. (18 points; 6 points each)

An amino acid whose R group you might expect to see bearing a positive charge at pH 8.



An amino acid that contains nitrogen, drawn at pH 3.3.

Any amino acid contains nitrogen!! All ionizable groups except α-COOH will be protonated at this pH.

An amino acid that you would expect to see in the fatty acid-associated portion of an integral membrane protein, drawn at pH 7.

Possibilities include:

- **The bulky aromatics: Tyr, Phe, Trp**
- **The bulky aliphatics: Leu, Ile, Val**
- **Methionine**
- **Perhaps small amino acids such as Ala, Gly, and Thr**

All have pKa ≈ 2 for α-COOH (- at pH 7) and ≈ 10 for α-NH_2 (+ at pH 7)

5b.) Pick two of the amino acids you diagrammed above, and describe how you might separate them from each other using techniques that you have read about, are using in lab, or that you have used before in a lab at Denison or elsewhere. You may choose to perform your separation at any pH value(s) you specify. Also indicate how you could determine that you had successfully separated them. Answering this effectively may require creativity and insight! (7 points)

The possibilities are…nearly endless!!

6.) Amino acid structures.

a) Please draw the structure of **one amino acid that matches each of the following** descriptions. Label each with its name and one-letter code. Please indicate the approximate charge you would expect to find on **all** ionizable functional groups at the indicated pH. [For reference, commonly ionized functional groups and their respective pK_A values are: α-carboxyl, 2; α-amino, 10; Aspartic acid and Glutamic acid carboxyl, 4; Histidine imidazole, 6; Lysine ε-amino, 11; Arginine guanidino, 12.5.] (15 points; 5 points each)

An amino acid whose R group you might expect to see bearing a positive charge at pH 5.

Candidates:

- **Histidine (H): α-COO^- , imidazol$^+$, αNH_3^+**
- **Lysine (K): α-COO^-, ε-NH_3^+, αNH_3^+**
- **Arginine (R): α-COO^-, guanidino$^+$, αNH_3^+**

An amino acid that you would expect to be able to detect on the UV-Vis spectrophotometers in the biochemistry lab, drawn as you would expect it to appear at pH 3.3.

I expect:

- **Phenylalanine (F): α-COO^-, αNH_3^+**
- **Tryptophan (W): α-COO^-, αNH_3^+**
- **Tyrosine (Y): α-COO^-, αNH_3^+**

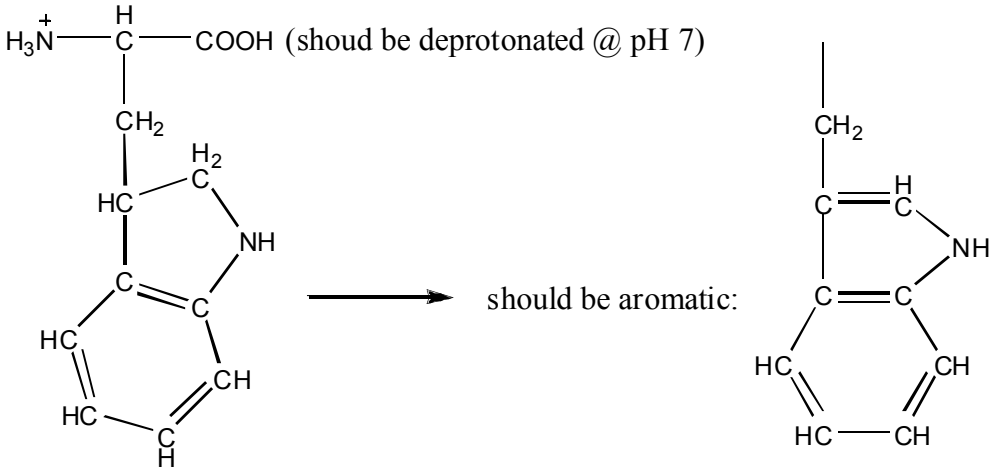
I will also eccept:

- **Asparagine (N): α-COO^-, αNH_3^+**
- **Glutamine (Q): α-COO^-, αNH_3^+**

An amino acid that you would expect to see exposed to solvent on the cytosolic face of an integral membrane protein, drawn at pH 7.

Candidates: any hydrophilic amino acid (e.g. S, T, N, Q, D, E, H, K, R, Y)

b) What is wrong with the structure drawn below if it is intended to show the amino acid tryptophan at pH 7? Please indicate any errors you find and draw the corrected structure. (5 points)

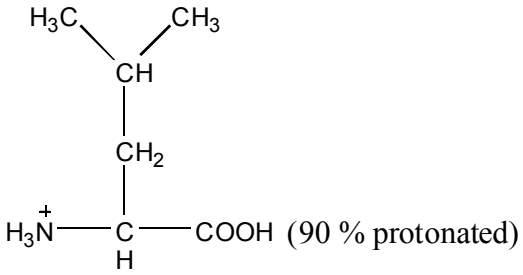


7.) Amino acid structures.

a) Please draw the structure of **one amino acid that matches each of the following** descriptions. Label each with its name and one-letter code. Please indicate the approximate charge you would expect to find on **all** ionizable functional groups at the indicated pH. You may round your approximate charges to the nearest 1/2. [For reference, commonly ionized functional groups and their respective pK_a values are: α-carboxyl, 3; α-amino, 8; Aspartic acid and Glutamic acid carboxyl, 4; Histidine imidazole, 6; Lysine ε-amino, 11; Arginine guanidino, 12.5] (15 points; 5 points each)

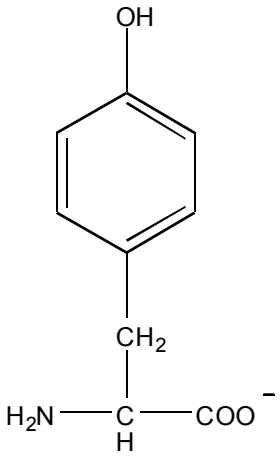
An amino acid that you would expect to see interacting with fatty acid tails on the membrane-exposed face of an integral membrane protein, drawn as a free amino acid at pH 2.

This should be a hydrophobic side chain, such as leucine (L):

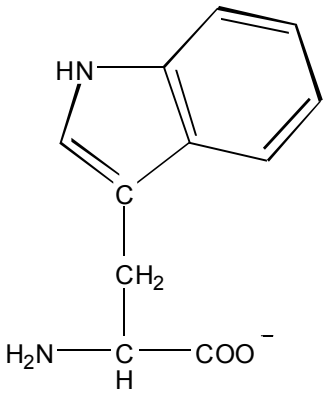


An amino acid that you would expect to be able to detect on the UV-Vis spectrophotometers in the biochemistry lab, drawn as you would expect it to appear at pH 12.5.

This should be an aromatic side chain, especially tyrosine (Y) or tryptophan (W):



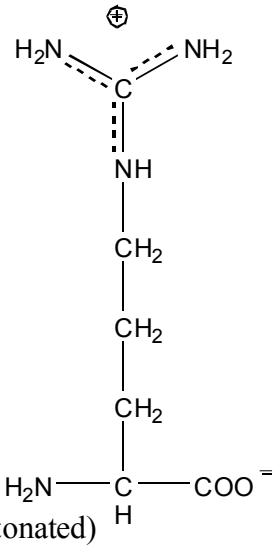
(Y)



(W)

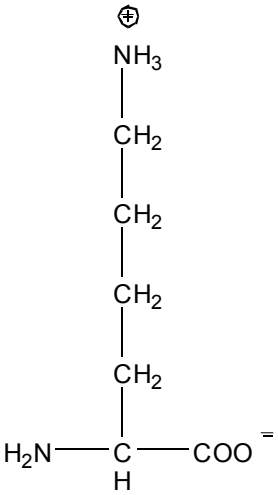
An amino acid whose R group you might expect to see bearing a positive charge at pH 9.

This can only be arginine (R) or lysine (K):



(90 % deprotonated)

(R)



(K)

8.) Amino acid structures.

a) Please draw the structure of **one amino acid that matches each of the following** descriptions. Label each with its name and one-letter code. Please indicate the approximate charge you would expect to find on **all** ionizable functional groups at the indicated pH. You may round your approximate charges to the nearest 1/2. [For reference, commonly ionized functional groups and their respective pK_a values are: α-carboxyl, 3; α-amino, 8; Aspartic acid and

Glutamic acid carboxyl, 4; Histidine imidazole, 6; Lysine ε-amino, 11; Arginine guanidino, 12.5] (15 points; 5 points each)

An amino acid that contains sulfur but does not form disulfide bonds, drawn as a free amino acid at pH 3.

This must be methionine, M. At pH 3, the alpha amine should be fully protonated (+1 charge) and the alpha carboxyl should be 50% deprotonated (- 0.5 charge).

An amino acid that releases ammonia when treated with strong acid, drawn as you would expect it to appear at pH 8.

This could be asparagine or glutamine, N or Q. At pH 8, their alpha amines should be 50% deprotonated (+ 0.5), and their alpha carboxyls should be fully deprotonated.

An amino acid that forms the basis of the protein quantitation assay you are using in lab, by binding to the dye Coomassie Brilliant Blue. [If you cannot remember which amino acids show special affinity for the dye, I will sell you that information for one point.] Please show this structure as you would expect it to appear in the buffer you are using for your protein assays in lab.

This could be any basic or aromatic amino acid: R, K, F, Y, or W. At the pH of your buffer in lab, pH 7, basic side chains will be fully protonated (+1), alpha amines will be almost entirely protonated (charge of about +0.9), and alpha carboxyls will be deprotonated (-1).

Size Matters:

1.) In 1996 a research group from NASA reported that a meteorite contained several types of evidence consistent with the hypothesis that there is (or has been) life on the planet Mars. One of the lines of evidence that the authors presented in their paper concerned microscopic wormlike structures that could be interpreted as the fossilized remains of bacteria. Due to their size (less than or equal to about 100 nanometers), these putative bacteria were termed "nanobacteria". Many other investigators have suggested that both theoretical arguments and observations of known life forms on earth make it extremely unlikely that life as we know it can occur in cells with diameters less than 0.2 microns (micrometers). On the other hand, geologist Robert Folk at the University of Texas, and some few others claim to have evidence for what they call "nannobacteria" that are as small as 0.01 microns in diameter. From what you know about the size of biological and biochemical structures, please comment on whether a cell 0.01 microns in diameter appears to be consistent with life as we know it. Argue your case concisely but fluently, giving specific details and supplementary drawings as you see fit. (15 points)

1.) Known lipid bilayers are on the order of 30~40 Å thick (~ 15 carbon fatty acids in lipids). Since a cell is a membrane-encased space, it will have (at least) two membrane thicknesses. This is already 6~8 nm, or 0.006~0.008 µm.
2.) Known life forms use ribosomes for translation, and known ribosomes are ~ 50 nm in diameter. Thus, a cell 0.01 µm across would only have room for one uncommonly small ribosome or similarly-sized protein. And 10 nm diameter proteins are not at all uncommon.
3.) Other arguments about minimal necessary DNA, protein, and raw materials for growth lead to the 0.2 µm number, but even just (1) and (2) above are enough to make me very skeptical about the viability of a 10 nm cell.

2.) In lab this week, you partially purified the protein cytochrome *c* from yeast cells by low-speed centrifugation and cation-exchange chromatography. Suppose that an absent-minded researcher were to mix a yeast lysate with his cation-exchange resin without first performing the centrifugation step. He would thus have a messy mixture of whole and broken yeast cells, glass beads, ion-exchange resin beads, and soluble cell components all floating around in a flask together. Please imagine that you could dive down into this mess and look around. What would you expect to see? Please sketch out a small portion of what this mixture should look like, being sure to include the following components: a cytochrome *c* molecule (MM 15,000), a yeast cell, a glass bead-beater bead, a bead from the cation exchange resin, and a sodium ion. Please include a rough scale bar in your sketch and indicate whether any of the components are either too big or too small to see at the level of magnification that you have chosen. (Artistic merit and structural accuracy will not be evaluation criteria here! 10 points)

Actual depiction will vary, of course, but…
- **Glass beads are easily resolvable by the naked eye (≤ 1 mm)**
- **Cation-exchange beads are smaller, appearing by eye more like powder (~ 50 µm)**
- **Yeast cells are a bit smaller, on the order of 10 µm (~ 5 µm)**
- **Cytochrome c is quite a bit smaller, on the order of 10 Å (4 nm)**
- **A sodium ion is smallest, with a radius of ~1 Å (100 pm)**

Ribosomes are the cellular site of protein synthesis, enzymatically producing polypeptides that range from a few thousand to a few hundred thousand daltons in size. These polypeptides are flexible polymers that must fold into complex three-dimensional shapes (though interactions

between their constituent amino acids, and between the amino acids and solvent molecules) in order to achieve biological activity. It has been proposed that the folding of most polypeptides into their final active conformations largely takes place while they are being synthesized on the ribosomes. By analogy, this would be like all the pieces of an automobile being synthesized in series off an assembly line, and spontaneously self-assembling into a functional car by the time the last part had come off the line.

Based on what you know about the relative amounts of time required for various biochemical processes, please tell me whether -- solely from the perspective of the time required -- you think this hypothesis of protein self-folding on the ribosome is feasible. Your answer should include a rough estimate of how long the protein synthesis process takes and should refer to other relevant biochemical processes.
(6 points)

Relevant data:
Protein synthesis takes a few seconds to occur, up to a minute for big proteins.
The individual enzymatic steps in protein synthesis take μsec to msec.
Molecular motion occurs in the time range of psec to nsec.

Logic:
Given those data, in the amount of time needed to make a protein, there is time for lots of movement: on the order of 10^9 movements. Even allowing for a highly complex series of conformational changes in the process of folding, and lots of dead-ends in the folding process (i.e., unstable conformations that have to be unfolded and tried again), there is a fair bit of time. This IS feasible.

Biomolecular Structure/Function Grab Bag:

1.) *Sulfolobus acidocaldarius*is a credit-card-shaped archaebacterium that lives in extremely low pH (pH ~2) conditions in Yellowstone National Park. *Thermotoga maritima* is a rod-shaped eubacterium that lives in high-temperature (> 80 C) thermal vents on the ocean floor. *Eurycea longicauda* is a salamander-shaped eukaryote that lives in my cellar under conditions that are, by comparison, rather boring and mild.

A. (6 points (+3)) What differences would you expect to find in the biochemistry of the two organisms that live in extreme conditions relative to the organism that lives under mild conditions, in terms of (1) the effect of pH on amino acids, (2) the effect of temperature on lipid composition, and (for extra credit) (3) the effect of temperature on nucleic acids?

1.) Low pH will cause R groups of charged amino acids in proteins to be protonated. Thus an organism that lives at pH ~ 2 cannot use negatively-charged R-groups without creating special pockets of altered pH. This will affect both catalytic mechanisms and intramolecular bonding mechanisms relative to proteins that function at pH ~ 7. Moreover, an organism that lives at pH ~ 2 is likely to have special mechanisms for protecting amino acids and proteins from acid-catalyzed hydrolysis.

2.) Membrane lipids in organisms that live at high temperature must be longer and more saturated than those in mesophilic organisms in order to reduce membrane fluidity. (In most cases, other compensatory mechanisms will also be involved.)

3.) Nucleic acids from organisms that live at high temperature contain typically much higher levels of G-C base pairs than DNA from mesophiles, consistent with what we would predict – a need to hold DNA and RNA chains together at high temperature.

B. (4 points) In spite of their differences in habitat, morphology, and physiology, I would argue that these three organisms, like all other organisms on earth, are descended from a common ancestor. Give me one piece of biochemical evidence which would support my position. Or, for the bold, one that would argue against it!

FOR:

- **All use the same types of polymers – sugars, nucleic acids, proteins, lipids – for the same purposes: energy, information, catalysis, and membranes.**
- **All share a sugar-based energy metabolism.**

AGAINST:

- **They use different kinds of lipids.**
- **They use different gene expression apparatus.**

2.) (5 points) Imagine two membranes, identical in composition except for the fact that one contains cholesterol and the other does not. If the T_m of the membrane WITHOUT cholesterol is 20 C, then which membrane would you expect to be more fluid at 37 C?

Cholesterol REDUCES fluidity above the T_m; the membrane without cholesterol will be more fluid.

(2 points) Since only animals make use of cholesterol in their membranes, could a plant achieve the same effect by using phophatidyl inositol containing two long, saturated fatty acids?

Long, saturated fatty acids should INCREASE the T_m, and DECREASE the fluidity. Thus, under a limited set of conditions, YES, this will be an adequate substitute. Below the T_m, however, this would have the opposite effect of cholesterol.

In other words, if your justification is correct, I will take either answer.

3.) Organism X has a lipid composition in its cell membranes that yields a T_m of 23 C. Its close relative, organism Y, has membranes with a T_m of 38 C.

a) Please suggest and defend two differences you might expect to find between the lipids from membranes of X and Y. (10 points)

X has a lower T_m. Thus, it might have either fewer saturated FA/shorter FA chains than Y. Both of these would decrease the T_m, as they increase fluidity at any given temperature.

b) Would you expect that the differences you identified in part (a) have an impact on the structure of integral membrane proteins from the two organisms? Why or why not? (5 points)

Although it is unlikely to have a huge effect for this T_m difference, a decrease in length of lots of FA could actually decrease the thickness of the membrane, requiring that the hydrophobic, membrane-spanning portion of integral membrane proteins be shorter as well.

c) Please suggest and defend one difference you would expect to find between homologous genes from X and Y. (5 points)

Organism X is apt to live at a lower temperature than organism Y, thus requiring the shift in lipid composition that leads to lower T_m. In order to effectively separate the strands of its DNA (at this lower temperature) for transcription and replication, X will likely want fewer H-bonds between base pairs. This will require a shift towards more A-T base pairing and fewer G-C base pairing.

4.) My wife and I live in an old farmhouse that we are in the process of partially rebuilding. As a result, the insulation in places...well...leaves a bit to be desired. This makes some parts of the house a bit chilly in the winter, a situation made worse our first winter there by the fact that we were primarily using a small wood-burning stove for heat. During long cold spells that first winter, the back porch barely stayed above 40 F/5 C. Quite a contrast with the situation in summer, when the non-air-conditioned porch could get close to 90 F/35 C.

If a cold-blooded animal (like a spider) lived on the porch year-round, you can well imagine the physiological adjustments they'd need to make in order to keep their biochemical machinery functioning properly under both of those temperature extremes. Consider especially the need to maintain a constant level of fluidity in their cell membranes in order to permit proteins to move within the membranes at the proper rate. Suppose the lipid composition of our spider's cell membranes during the summer heat wave was:

Percent lipids as phospholipids:	70%
Percent saturated fatty acids:	80%
Average fatty acid chain length:	18 carbons

a) Give or take 5 degrees C, what would you expect the phase transition temperature (T_m) of these membranes to be during the summer months? Why? (3 points)

To maintain some level of fluidity, T_m should be a few degrees below ambient T – say T_m to be approximately 30°C.

b) What changes might you expect to see in the lipid composition values as the spider became acclimated to living on the cold porch during the winter months? Please list two possible changes and **justify each one rigorously**. (12 points)

As $T_{ambient}$ goes from 35°C to 5°, possible changes need to reflect a decrease in T_m in order to maintain fluidity. Examples:

i. **Increase % unsaturation from 20% to 40% or so. Increasing unsaturation will fairly dramatically increase fluidity as it greatly interferes with FA-FA associations and packing in the membrane due to kinked shape.**
ii. **Reduce average chain length from 18 carbons to … possibly 14. Decreasing chain length, again, interferes with inter-FA associations and decreases stability of packing, thus increasing fluidity.**

5.) Biomolecular structure/function grab-bag. For each of the following situations, please answer the question in one or a few short sentences. Please remember to justify each of your answers. (4 points each)

a) One of my cats, Picchu, has a passion for lettuce, spinach, tomatoes, and melon. Thinking about Picchu's unusual diet leads me to imagine a cat that desires to eat *only* fruits and vegetables, and is furthermore genetically unable to synthesize cholesterol. Suppose that such a mutant vegetarian cat were to live in a very hot environment. What differences would you expect to find in its membrane lipids relative to a mutant vegetarian cat living at a much cooler temperature?

High temperature vs. low temperature – more saturation, longer FA chains.

b) True or false: at a pH equal to its pK_A, the average charge on an ionizable amino acid side-chain (such as lysine's ε-amino group) will be zero.

FALSE: at pH=pKa, 50% ionized, so either net +1/2 or net -1/2.

c) In his extra-credit buffer question/prediction, one of your classmates mentioned "*Picrophius oshimae* …[t]his organism is the most acidophilic prokaryote known and it is also a thermophile. It grows optimally at pH 0.7 and at 60˚C." If this description is correct, please predict whether you would expect the DNA of this organism to contain a HIGHER or LOWER fraction of total nucleotides as As and Ts than the DNA of *Salmonella,* which has an optimal growth temperature corresponding to the temperature of the human intestinal tract, 37 C.

High temperature vs. low temperature – more G-C base pairs, thus lower number of A-T base pairs.

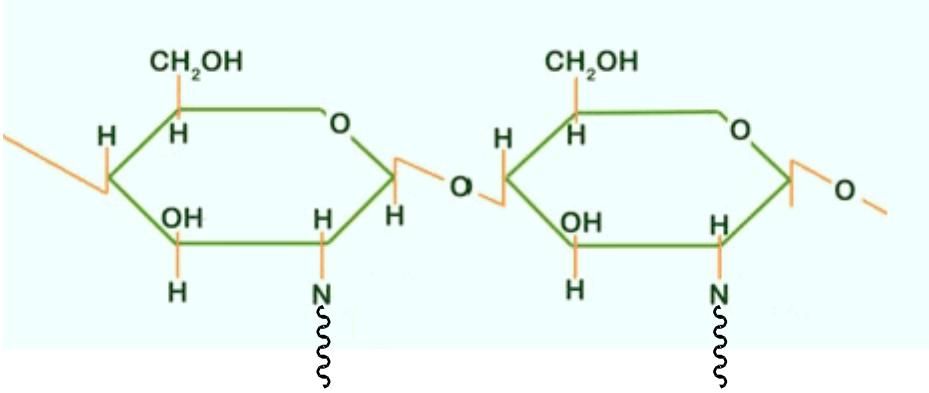
d) Please briefly tell me one reason why an organism living at pH 0.7 would have to have evolved novel ways of replicating its DNA.

At pH 0.7, proteins will have very few - charged AAs. Even very acidic nitrogens in NA bases will be protonated. These factors will change protein-protein, nucleotide-nucleotide, and DNA-protein interactions and will necessitate a lot of custom features.

e) To my mind, the available biochemical evidence suggests that all known life forms have evolved from a (ancient) common ancestor. Please briefly describe one piece of evidence that is either (i) consistent with, or (ii) inconsistent with, this statement.

Lots of possibilities here…

f) Chitin is a polysaccharide that is the major constituent of arthropod exoskeletons. It is a homopolymer composed of β-1,4-linked N-acetyl glucosamine monomers (glucose molecules whose 2-hydroxyl group has been replaced by an N-acetyl group). Please draw a close-up of the glycosidic bond in chitin.



6.) Biomolecular structure/function grab-bag. For each of the following situations, please answer the question in one or a few short sentences. Please briefly justify each of your answers.

a) Suppose I were to take a cryophilic bacterium that lives in the snow in Antarctica, and a hyperthermophilic bacterium that lives in a hot-spring in Yellowstone National Park, and instantaneously transfer them both to containers of nutrient broth at room temperature over in the microbiology lab in Talbot Hall. Which organism would you expect to display a **higher membrane fluidity**? (3 points)

The cryophile needs higher fluidity to maintain cell membrane mobility @ very low temperatures. At room temperature ($T_{room} >> T_{snow}$), it would be very fluid. For the thermophile, $T_{room} << T_{hot\ spring}$, and it is likely to be very stiff.

b) Which of the bacteria in part (a) would you expect to utilize a **higher proportion of saturated fatty acids** in its membrane lipids under normal growth conditions? (3 points)

Saturated FA decrease fluidity. I would expect to see more of them in the high-temperature organism.

c) True or false: at a pH equal to its pK_A, the average charge on an ionizable amino acid side-chain (such as lysine's ε-amino group) will be zero. (4 points)

FALSE – At pH=pKa, charge will be -0.5 or +0.5.

d) Briefly explain why starch [poly-(glucose-α(1,4)-glucose)] and cellulose [poly-(glucose-β(1,4)-glucose)] have different physical shapes and different physical and biological properties even though they have the same chemical composition: $(C_{12}H_{21}O_{11})_n$
(4 points)

The geometry at the glycosidic bond – the anomeric carbon – differs. This affects overall geometry. It also affects susceptibility to enzymatic action at the anomeric bond.

e) Persons who suffer from inherited metabolic disorders such as PKU, gout, or hemochromatosis can attribute their symptoms to defective enzymes in their cells. Please explain briefly how a systemic protein defect can be inherited. (3 points)

We inherit DNA, which encodes proteins. We thus inherit instructions for protein sequence and structure.

f) The cation exchange resin that you used in lab this week is derivatized with a carboxylic acid group that has a pK_a of about 4. Suppose that you were trying to purify a positively-charged protein that is most active at pH 3. Would you be able to use the BioRex resin for purifying this protein (at pH 3)? (3 points)

pH 3 < pKa. Therefore, 90% of resin will be protonated and hence neutral. This will not work well for purifying a charged protein. Bummer ☹

7.) Biomolecular structure/function grab bag (20 points; 4 points each).

Shrimp, lobster, and crayfish feature prominently in creole cuisine. Think shrimp jambalaya, crayfish etouffe, or a rich seafood gumbo. Of course, to make these dishes, one needs to catch some crustaceans, or at least to buy them at the store. The Northern Pink Shrimp, *Pandalus borealis*, is harvested commercially at moderate depths in the northwest Atlantic, and it is commonly found at temperatures of 2~8 ^oC. The dominant species caught in the Gulf of Mexico, on the other hand, are White and Brown shrimp (*Penaeus setiferus* Linnaeus and *P. aztecus* Ives, respectively). These species prefer near-shore and estuarine waters and thrive when water temperatures rise above 20 ^oC. Given these differences in habitat, please answer the following three questions.

a) Would you expect Northern Pink shrimp or Brown shrimp to **have a higher level of saturated fatty acids** in their membrane lipids? Please explain your answer in a single sentence.

I would expect the Brown shrimp to have a higher level of saturated fatty acids, as they need to retain lipid structure at higher temperatures than the Northern Pinks.

b) If you extracted membrane phospholipids from Northern Pink and Brown shrimp, and reconstituted them as lipid vesicles in water, which would you expect to **show greater fluidity at 15 ^{o}C**?

I would expect the Northern Pink shrimp lipids to be more fluid at this temperature, as it is above their normal living temperature, but below the optimal temperature for the Browns.

c) Purely on the basis of the available data, would you expect to find any **differences in the base composition** (amounts of A, C, G, and T) of DNA from Northern Pink versus Brown shrimp? If so, what are they? Please explain briefly.

Purely on the basis of these data, I'd expect that the Brown shrimp would have a higher fraction of Gs and Cs than the Northern Pinks, in order to maintain DNA double-stranded-ness at their higher living temperatures.

d) MSG, monosodium glutamate, is a flavor enhancer that is commonly added to many dishes, and even to packaged meat and seafoood. The mechanisms by which MSG exerts its flavor enhancement effects and its occasionally severe side effects are fascinating biochemical tales that we can't get into here (bummer, eh?). You should recognize, however, that MSG is simply the monosodium salt of the common amino acid glutamic acid. Given what you know about glutamic acid, what would you predict to be the relevant pK_a for the dissociation of MSG in aqueous solution?

Glutamic acid has three pKa values corresponding to the deprotonation of its three ionizable functional groups. These are ~3 for the alpha-carboxyl, ~4 for the side-chain carboxyl, and ~8 for the alpha amine. If MSG is singly deprotonated, than the most acidic hydrogen is gone, and the next proton to go will be the side-chain carboxyl proton, with a pKa of ~4.

e) Cooking breaks down biomolecules and supramolecular structures, changing the flavor and texture of foods. Of the following kinds of intermolecular interactions, which would you expect to be most heat labile: disulfide bonds in proteins, ionic interactions between components of mitochondrial membranes, or hydrogen bonds in DNA?

The weakest of these interactions, and therefore the most likely to be broken by the heat energy of cooking, is the hydrogen bond.

8.) Fundamental information. The following questions probe concepts that you have learned in prerequisite courses and are important for a basic mastery of Biochemistry. No explanations are necessary. (15 points; 3 points each)

a) What is a gene? What are the basic elements of **any** protein-coding gene's structure?

A gene is the fundamental unit of genetic information and biological function. It is a chunk of DNA that encodes an RNA or a protein. All protein-coding genes must contain transcriptional start (promoter) and stop sequences and translation start and stop sequences and ORF.

b) Which is of longer duration: a picosecond or 100 nanoseconds?

100 nanoseconds MUCH longer; it is equivalent to 10^5 picseconds.

c) If I want to make 30 mL of a 0.6 M solution of sodium hydroxide (40 g/mol), how many grams of solid NaOH must I dissolve in water?

0.030 L sol'n × 0.6 mol NaOH/L sol'n × 40 g NaOH/mol = 0.72 grams

d) Suppose that I combust a series of jelly beans under controlled conditions, and monitor how much heat is given off in each experiment. I find that, on average, my jelly beans give off 50.0089876 kilojoules of energy when completely burned. The 95% confidence interval on this average is remarkably small, 0.0004563 kilojoules, indicating very high quality control by the jelly bean manufacturer. How should I report the average heat value of my jelly beans (+/- the CI), if I am being careful about my significant figures and units?

50.0090 +/- 0.0005 kJ *(always round the measure of uncertainty, the CI, to one sig. fig., then round the average to the same number of decimal places)*

e) In which of the following compounds is the carbon most reduced (electron-rich): methane (CH_4), methanol (CH_3OH), formaldehyde (H_2CO), or formic acid (HCOOH)?

Carbon is most reduced (least oxidized) in methane.

4. ***Interactions between molecules in biochemical systems.***

a) Please rank the following interactions in terms of **increasing bond strength**:

i. the interaction that holds the phosphate portion to the glycerol portion of a single phospholipid molecule **THIS IS A COVALENT LINKAGE**

ii. the interaction that holds the fatty acid portion of one phospholipid to the fatty acid portion of an adjacent phospholipid molecule **THIS IS MEDIATED BY DISPERSION FORCES**

iii. the interaction that holds an amino acid side chain like lysine in an integral membrane protein to the phosphate head-group of a phospholipid in the membrane in which the protein is embedded **THIS IS AN IONIC INTERACTION**

(4 points)

Covalent is stronger than ionic (on average) is stronger than dispersion, so
ii < iii < i
Note, however, that the aggregate effect of LOTS of dispersion force interactions serves as a very effective way to hold the membrane lipids together.

Another way to think about this is: if you imagine shaking a cell harder and harder and harder, in what order would these things come apart? Certainly the last to come apart would be the bonds within individual molecules (i); ii and iii may be pretty close in actual effect.

b) Avocados are about 15% lipids by mass, of which about two-thirds contain mono-unsaturated fatty acids. They are grown primarily in sub-tropical regions; in our region, Mexico and southern California are the main avocado-growing areas.

Suppose that an enterprising crop scientist decided to breed avocados that would thrive in Alaska, with long hours of summer sunlight but much cooler temperatures than traditional avocado-growing regions. As discussed in class, the environment in which an organism lives is often reflected in differences in its biomolecules relative to closely-related organisms that live in different environments. Please speculate about the differences you'd expect to see in the lipid composition of Alaskan avocados when compared with sub-tropical Mexican avocados. Please be as specific as possible, and be sure to justify your predictions based on the physicochemical properties of the different lipids.
(7 points)

At the lower temperatures expected in Alaska, the northern avocados will still need to maintain adequate lipid fluidity to permit normal biological function. Therefore, it is not unreasonable to expect changes that would decrease the T_m of the avocado lipids. In general, these will be changes that DEcrease interactions between fatty acid tails by inhibiting dispersion force interactions. In specific, it'd be reasonable to expect to see shorter FA tails and more unsaturation in the northern fruits.

c) Organization of biomolecules into biologically active structures -- for instance, the folding of polypeptides or the formation of lipid bilayers -- requires interactions both between the biomolecules and solvent molecules and between the portions of the biomolecules themselves. Some of these interactions are primarily entropically-driven, and some are driven by favorable enthalpy changes. Please identify each of the following interactions as being driven primarily by favorable **entropy** or **enthalpy**:

the association of bulky aliphatic amino acids in the core of a protein __**entropy**__
(a case could be made for attributing this to enthalpic forces. If you wrote "enthalpy" and I marked it as incorrect, you are welcome to try to justify your answer to me for a revised score.)

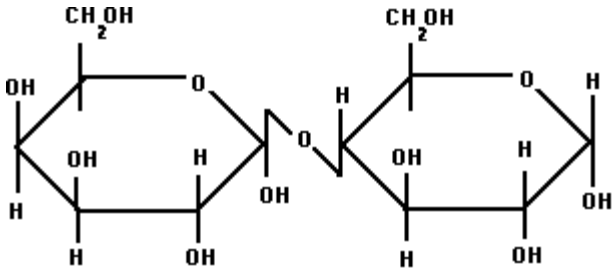
the association of water with polar amino acids on the outside of a protein _**enthalpy**__

<u>Other Structures:</u>

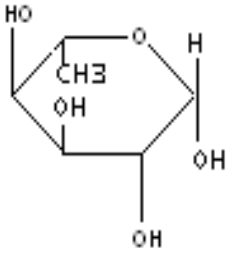
1.) Monotremes use the monosaccharide fucose as their milk sugar; eutherian mammals (like us) use the disaccharide lactose as theirs.

A. (5 points) On the structures of these two sugars below, please label:
* the names of the two sugars
* the glycosidic bond; which carbons does it connect? What is the orientation of the side chains at these positions?

* the rather unusual 6-deoxy "group" on fucose



Lactose

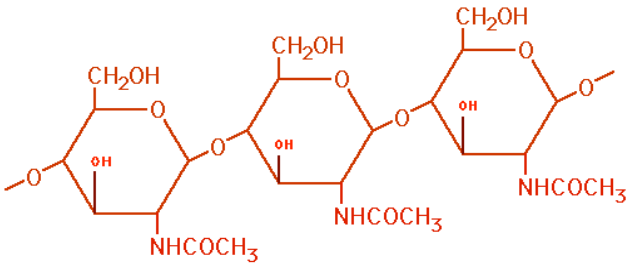


Fucose

B. (3 points) The inability of some individuals to digest lactose is called "lactose intolerance" and leads to digestive distress as the result of undigested sugar accumulating in the gut. This condition stems from the reduced expression of an enzyme that breaks a particular kind of bond. Which bond in lactose do you think is the problem (and why)?

As much as mammals lack an enzyme that will catalyze the hydrolysis of the glycosidic bond in cellulose, many have developmentally-regulated expression of the enzyme needed to catalyse the hydrolysis of the glycosidic bond in lactose.

2.) Cellulose and starch are both composed exclusively of repeating glucose units, strung together by (1-4) glycosidic bonds. However, although our bodies use glucose for energy, and indeed we can hydrolyze the α(1-4) glycosidic bonds in starch to generate metabolic fuel, we have no enzymes capable of hydrolyzing the β(1-4) linkages in cellulose and so it is, for us, metabolically inert. Below is a depiction of the basic repeating structure of chitin, a polysaccharide that forms the bulk of the tough exoskeleton of arthropods. Chitin is built from N-acetyl-glucosamine monomers, essentially glucose molecules modified at the C-2 position.



My friend Jim is a big fan of exotic foods. If I had taken advantage of the plentiful natural resources available to me this past spring and made Jim a steaming platter of stir-fried cicada skins, would he likely have been able to derive much metabolic energy from the chitin? (11 points)

Unfortunately, chitin is composed of monosaccharides linked by β(1→4) glycosidic bonds. Therefore, it, like cellulose, is undigestible by humans. Sorry Jim, nice taste but no energy ☹.