Biochemistry I - Exam Face Page

- **This exam consists of 9 pages, including this one.**
- **There are a total of 90 points - or approximately 2 points /minute of exam time.**
- **Many questions have choices, if you answer more than one of the choices, your best answer will be counted.**

The following equations and constants may be useful:

T=300K and pH=7.0 unless otherwise stated.

R=8.3 J/mol-K   RT=2.5 kJ/mol @ 300K

Log2=0.3   ln10=2.3

T(K) = T(C) + 273.

Thermodynamics:

$$\Delta G^0 = -RT\ln K_{eq}$$

$$\Delta G = \Delta H - T\Delta S$$

$$S = R\ln W$$

$$\ln(a^n) = n \ln a$$

For the reaction: $N \leftrightarrow U$:

$$K_{EQ} = \frac{[U]}{[N]}$$

$$f_U = \frac{K_{EQ}}{1+K_{EQ}}$$

$$f_N = \frac{1}{1+K_{EQ}}$$

Acid-Base & Buffers

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$

$$R = 10^{pH-pK_a}$$

$$[HA] = [A_T]\frac{1}{1+R}$$

$$[A^-] = [A_T]\frac{R}{1+R}$$

Beer's law:

A=ε[X]l

$\varepsilon_{\lambda=280} = 5,000$ for Tryptophan (Trp)

$\varepsilon_{\lambda=280} = 1,500$ for Tyrosine (Tyr)

Ligand Binding:

For [M]+[L] ↔[ML]

$$K_{EQ} = \frac{k_{on}}{k_{off}}$$

$$\theta = \frac{[ML]}{[M]+[ML]} = \frac{[L]}{K_D+[L]}$$

Amino Acid Names:

Alanine: Ala
Arginine: Arg
Asparagine: Asn
Aspartic Acid: Asp
Cystine: Cys
Glycine: Gly
Histidine: His
Isoleucine: Ile
Lysine: Lys
Leucine: Leu
Methionine: Met

Phenylalanine: Phe
Proline: Pro
Serine: Ser
Threonine: Thr
Tryptophan: Trp
Tyrosine: Tyr
Valine: Val
Glutamine: Gln
Glutamic Acid: Glu

1: _____/ 9

2: _____/ 6

3: _____/12

4: _____/ 8

5: _____/12

6: _____/ 6

7: _____/11

8: _____/14

9: _____/12

TOTAL: _____/90

1. (9 pts) A titration curve of an amino acid is shown on the right. Please answer the following questions.

pH Titration

   i) What are the pK_a values associated with this amino acid? Briefly explain how you got these values (3 pt).

   The pK$_a$ values are 2, 6 and 9. (1 ½ pt)

   These were obtained from the three inflection points on the titration curve (1 ½ pts)

   ii) Mark **one** pH range on this curve where this amino acid would serve as a buffer. *Briefly* justify your answer (3 pts).
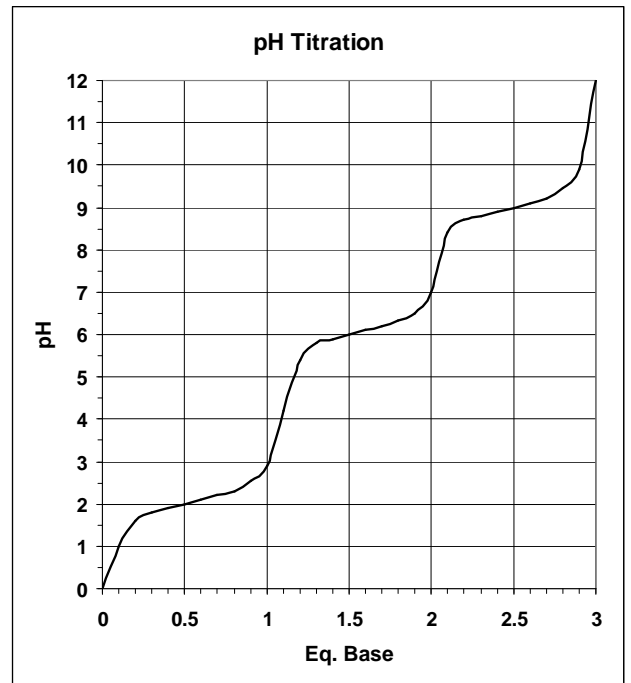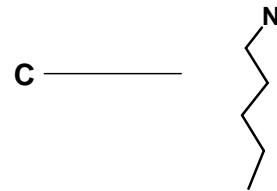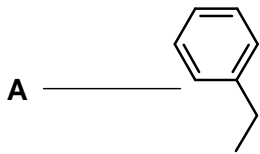   Any of the pKa values can be used +/- 1 pH unit:
   1-3
   5-7
   8-10
   (However, my titration as drawn suggests a slightly narrower range)

   iii) Select from the choices of *sidechains* below, the amino acid that would most likely produce the above titration curve. Write the approximate pK_a for its sidechain next to its structure on the diagram below. Give the name of this amino acid (3 pts).
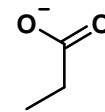
A ———

B ———

C ———

   B is the correct answer– histidine, with a pKa of 6.0. The pKa at 2.0 is the carboxyl group, the pKa at 9.0 is the mainchain amino group. A (phenylalanine) is incorrect because it has no ionizable group on its sidechain. C (lysine) is incorrect because the pKa of its sidechain is 9.0.

2. (6 pts) The pK_a of the carboxyl group in the *sidechain* of aspartic acid (Asp) is 4.0. In alanine (Ala), the pK_a of the *mainchain* carboxyl is 2.0. Please answer either of the following **two** choices. Circle your choice.

Aspartate sidechain   Alanine

   Choice A: When the Asp sidechain in a protein is close to an amino group, its pKa usually decreases, why?
   A smaller pKa indicates a stronger acid, i.e. the deprotonated state is energetically more favorable. In this case the negative charge on the deprotonated Asp is stabilized by the positive charge on the amino group.
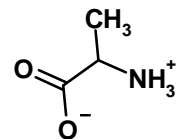
   Choice B: When the Asp sidechain in a protein is close to another carboxylate group, its pKa usually increases, why?
   A larger pKa indicates a weaker acid, i.e. the deprotonated state is energetically less favorable. In this case the negative charge on the deprotonated Asp is destabilized by the negative charge on the carboxylate group.

3. (12 pts) Select **one** of the following four interactions or effects. *Briefly* describe the molecular nature of your choice, state whether it is related to the entropy or enthalpy of the system, and then describe its role in the stabilization (or destabilization) of typical globular proteins. Rank the importance of your choice in stabilizing/destabilizing the folded form of the protein relative to the other three choices.

Sample answer: Electrostatic effects involve attraction and repulsion of charged groups in proteins (e.g. Asp and Lys). This is largely an enthalpic (ΔH) effect. It has very little influence on stabilizing either the folded or unfolded form of the protein, all other effects are more important.

    i) the hydrophobic effect   iii) van der Waals forces
    ii) hydrogen bonds   iv) Conformational entropy

i) the hydrophobic effect is the ordering of water molecules around non-polar groups that become exposed during unfolding (4 pts). It is entropic (+4 pts) and it is the most stabilizing factor for the folded form of the protein (+4 pts).

ii) Hydrogen bond involve the donation of a proton from an electron negative atom to another electronegative atom, i.e. N-H   O= (4 pts). This is largely an enthalpic term (+4 pts). It stabilizes the folded form (+2 pts), but only weakly (+2 pts).

iii) van der Waals forces involve induced dipole-dipole interactions between any molecules (+4 pts). This is an enthalphic effect (+4 pts) and they stabilize the folded form of the protein (+2 pts). They are more important than hydrogen bonding (+2 pts).

iv) Conformational entropy is the entropy gained by the large number of conformations that the polypeptide chain can assume when it unfolds (+ 4pts). It is obviously and entropic term (+2 pts, a gift) and can be approximated as $S = R \ln W = R \ln (9)^N$ (+ 2pts). It is very destabilizing for the folded form of the protein (+4 pts).

4. (8 pts) Describe how an α-helix is similar to a β-sheet (or β-hairpin). How do they differ? (A well labeled diagram is an acceptable answer).

    Similarities (+ 4 pts):

      Both are secondary structures that are stabilized by hydrogen bonds.

      Both are conformations with phi and psi angles that minimize steric clashes.

    Differences (+ 4 pts):

      A helix is helical in shape with H-bonds || to the helix axis, side chains project out.

      A strand is extend with hydrogen bonds between the strands (perpendicular to the strand direction)

5. (12 pts) Draw, in the space below, *any* dipeptide that contains two *different* residues (Yes, you can use the various amino acid structures found in this exam). In your drawing, the peptide bond should be *trans* (**+4 pts for a completely correct drawing, -1 if your peptide bond was not trans**). On your drawing indicate:
   i) The peptide bond (1 pt).
   ii) The bonds whose torsional angles are defined by φ and ψ (order not important) (2 pt).
   iii) The name of your dipeptide (1 pts).
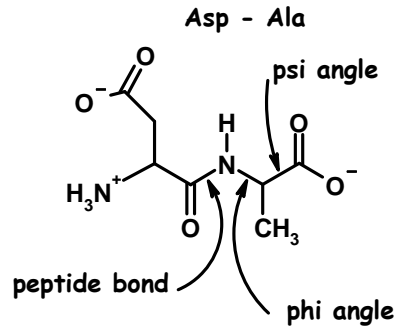
iv) Please answer any **one** of the following three choices (4 pts). Please circle your choice.
   Choice A: Which bond(s) in the dipeptide is/are planer and not free to rotate. Why is this so?
   Choice B: Why are most peptide bonds *trans*?
   Choice C: Briefly describe the Ramachandran plot and the origin (or source) of the low energy conformations that are contained within the plot.

A) The peptide bond is not free to rotate due to partial double bond character or conjugation of the nitrogen p_z orbital with the C=O orbitals.

B) The cis form leads to unfavorable van der Waals interactions between mainchain and sidechain atoms (steric clashes)

C) A point on the Ramachandran plot gives the phi and psi values for a residue. The low energy conformations are those that avoid steric clashes or, in other words, have favorable van der Waals interactions.

6. (6 pts) Please do either of the following **four** choices. Please circle your choice:
   Choice A: Briefly describe the term "quaternary structure". Provide an example of one.
   Choice B: What are super-secondary structures? Give an example.
   Choice C: Distinguish between the secondary and tertiary structure of a protein.
   Choice D: Describe the overall structure of an antibody molecule. Indicate where antigen binds on this molecule and the location of the Fab fragment.

A) The arrangement of multiple polypeptide chains. Hemoglobin and immunoglobulins are examples.

B) Super secondary structures are built from secondary structures. The β-α-β unit is one, the β-barrel is another.

C) The secondary structure only refers to the conformation of the mainchain atoms. The tertiary structure describes the location or conformation of both mainchain and sidechain.

D) Y-shaped. Two light chains. Two heavy chains. V-regions at beginning of light and heavy chain are where the antigen binds. The Fab fragment consists of the entire light chain and the V and 1st constant region of the heavy chain. A diagram was an acceptable answer.

7. (11 pts) A peptide that is 12 residues in length was subject to Edman degradation. Due to the small amount of the original peptide, only the first 5 residues could be unambiguously identified. The sequence obtained from these data is:

<div align="center">Gly-Ser-Arg-Phe-Phe</div>

The original peptide was then treated with the protease chymotrypsin, and the peptides or free amino acids that were produced from this cleavage reaction were sequenced to give the following data:

Gly-Ser-Arg-Phe,　Phe(free amino acid), and a peptide with the sequence: Ser-Thr-Lys-Leu-Met

plus one additional amino acid that could not be reliably identified.

i) Give as much of the original sequence as possible in the space below. The first five residues are already listed for you. *Briefly* justify your answer (5 pts).

Gly – Ser – Arg – Phe – Phe -_____-_____-_____-_____-_____-_____-_____
　　1　　2　　3　　4　　5　　6　　　7　　　8　　　9　　　10　　　11　　　12

> There are three possible answers, any were acceptable. Alternatively, you could have stated that it was not possible to come up with a unique solution (Note: The clarification given during the exam limited the solution to just A.)
>
> A) The partial sequence is: Gly-Ser-Arg-Phe-Phe-Ser-Thr-Lys-Leu-Met-X-Y
> B) The partial sequence is: Gly-Ser-Arg-Phe-Phe-X-Ser-Thr-Lys-Leu-Met-Y
> C) The partial sequence is: Gly-Ser-Arg-Phe-Phe-X-X-Ser-Thr-Lys-Leu-Met
>
> Where 'X' is either Phe, Trp, or Tyr (i.e. cleavage site for chymotrypsin) and Y is any other amino acid, including Phe, Trp, or Tyr.

iv) If you wanted to complete the sequence using another cleavage reagent, which would you use and why? (1 pts)

You could use either trypsin, which cleaves after Lys, or Arg. This would produce peptides that would complete the sequence for all three possibilities. Cyanogen bromide, which cleaves after Met, would complete the sequence of only the first solution, this would be acceptable if you just stated the 1st solution.

v) A 10 μM (1 μM = 10^{-6} M) solution of the peptide has an absorbance of 0.1 (assume path length of 1 cm). Based on this information, can you determine the 11th and 12th amino acids? How? [**Note**: Ignore the UV absorption of Phe in this problem, it's small anyway.] (5 pts)

$$A = \varepsilon[X]l$$
$$\varepsilon_{TRP} = 5{,}000\,M^{-1}cm^{-1}$$
$$\varepsilon_{TYR} = 1{,}500\,M^{-1}cm^{-1}$$

A= ε [X] l
Solving for ε:　ε = A/[X] = 0.1/10^{-5} = 10^4　= 2 ×ε$_{TRP}$.

Therefore the peptide contains two tryptophan residues. So the final possible solutions are:
A) Gly-Ser-Arg-Phe-Phe-Ser-Thr-Lys-Leu-Met-Trp-Trp;　the 11th and 12th aa are Trp and Trp.
B) Gly-Ser-Arg-Phe-Phe-Trp-Ser-Thr-Lys-Leu-Met-Trp;　the 11th and 12th aa are Met and Trp.
C) Gly-Ser-Arg-Phe-Phe-Trp-Trp-Ser-Thr-Lys-Leu-Met;　the 11th and 12th aa are Leu and Met.

8. (14 pts) Please select **one** of the following three choices. Circle your choice.

Choice A: The core of a globular protein consists entirely of six valine residues. The melting curve for this protein, as well as a number of variants is shown to the right. The thermodynamic parameters for these proteins are shown in the table below.
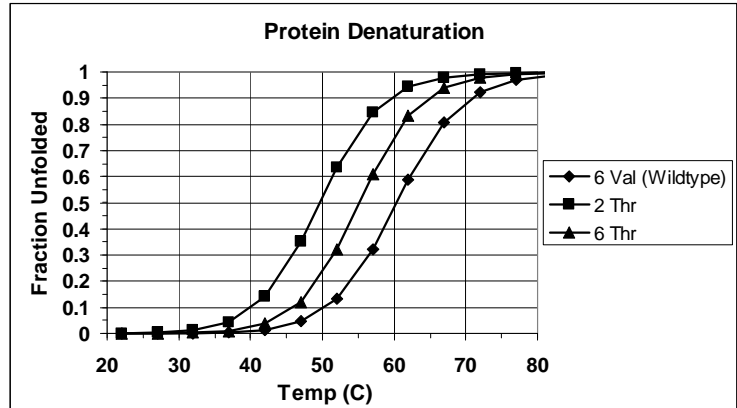

Protein	$\Delta H^{o}_{(N \to U)}$	$\Delta S^{o}_{(N \to U)}$
Native protein (6 Val)	+200 kJ/mol	+600 J/mol-deg
Variant 1 (2 Thr+4 Val)	+200 kJ/mol	+620 J/mol-deg
Variant 2 (6 Thr)	+210 kJ/mol	+640 J/mol-deg

i) Briefly describe how you would obtain the enthalpies from these denaturation curves (2 pts).

A plot of ln K_{EQ} versus $1/T$ would have a slope of $-\Delta H^o/R$.

K_{EQ} is obtained at different temperatures by reading the fraction unfolded off the graph: $K_{EQ} = f_U/f_N$

ii) Replacement of two of the valine residues with the amino acid threonine causes the protein to become much less stable than the original. Briefly explain which interactions have been affected by this replacement and explain why the protein is less stable. You should include the above changes in the thermodynamic parameters in your discussion. (**Note**: Pay close attention to the structure of the sidechains of valine and threonine when formulating your answer.) (6 pts)
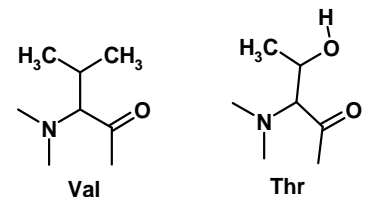

Valine and Threonine are very similar in size, therefore van der waals won't be affected too much. This is why the ΔH values are the same for both proteins (+ 6 pts, partial credit for a reasonable description that discussed enthalpic effects.)

Threonine is less non-polar (hydrophobic) than valine, so it orders less water molecules in the unfolded state. This is supported by the increase in the overall entropy. The negative entropy of the hydrophobic effect is smaller, so the reduction of the positive conformational entropy is higher. (+ 6 pts)

iii) Surprisingly, replacement of all six of the valine residues with threonine increases the stability of the protein somewhat, giving the middle denaturation curve in the above graph. Interpret this result using the thermodynamic parameters given in the table. (6 pts)

The internal threonine residues are forming hydrogen bonds with each other, as shown by the increase in the ΔH (+ 6 pts).

Q8 Choice B: The binding of aminobenzene to a Fab fragment was measured using equilibrium dialysis at two different pH values. The binding curves for each pH value are shown to the right. The structure of aminobenzene interacting with one of the amino acid residues (aspartic acid) from the antibody is shown below.

Aminobenzene Binding Curve
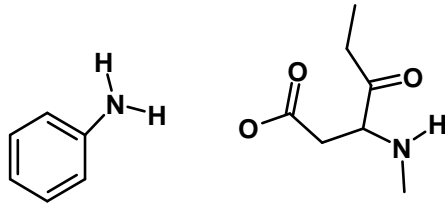
Aminobenzene   Fab Fragment
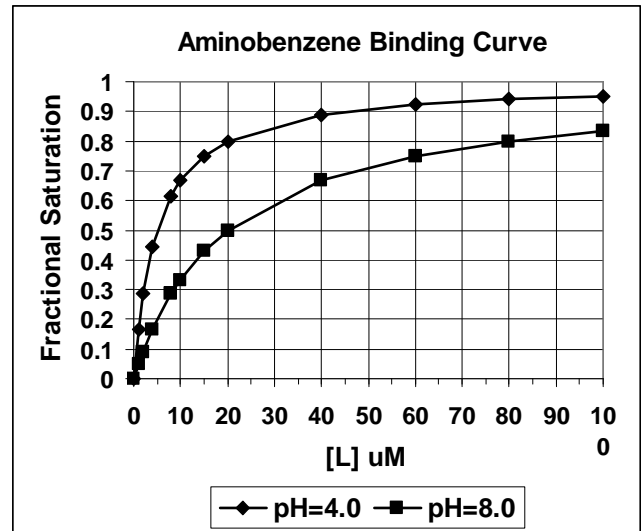
i) Determine the K_D for binding at each pH value.
  Enter you answer in the table to the right. Briefly justify your approach. (5 pts)

+3 pts for correct values.

The Kd values are the ligand concentration that gives θ=0.5.   (+2 pts)

pH	K_D
4.0	5 uM
8.0	20 uM

ii) At what pH value is the binding the strongest?   Justify your answer by reference to the K_D values. (5 pts)

At pH 4.0. A low Kd indicates stronger binding.

iii) Briefly explain the effect of pH on the binding affinity. Your answer should clearly indicate how the interaction between the aminobenzene and the Fab fragment changes as a function of pH and how this affects the measured K_D. You can assume that the pKa values of the aminobenzene and the sidechain of the amino acid from the Fab fragment are both 4.0 (4 pts).

At pH 4.0 the amino group on the aminobenzene will be 50% protonated (pH=pKa) and the Asp sidechain will be 50% deprotonated with a negative charge. The amino benzene binds well because of the electrostatic interactions (+ 3 pts)

At pH 8.0 the amino benzene is fully deprotonated and has no charge, therefore the binding is weak. (+ 1 pt)

Q8 – Choice C: A 60 residue globular protein unfolds with an enthalpy of +200 kJ/mol with a melting temperature of 333 K. Please answer the following questions:

$$\Delta G^o = \Delta H^o - T\Delta S^0$$
$$= -RT \ln K_{EQ}$$
$$R = 8.3 J/mol - K$$
$$S = R \ln W$$

i) Determine the overall *entropy* of unfolding (3 pts).

The entropy can be found by realizing at T_M, ΔG^o is zero:

$\Delta S^o = \Delta H^o/T_M$

= 200000/333 = 600 J/mol-K

ii) Estimate the change in *entropy* due to conformational changes when this protein unfolds (4 pts).

$\Delta S = R \ln (10)^{60} = 60 (8.31) \ln 10 = 1147$ J/mol-K
(using 9 for the average number of conformations of each residue was also correct.)

iii) Based on your calculated value for *part ii*, what is the contribution of the solvent entropy to the overall protein stability? (3 pts).

$\Delta S^0_{TOTAL} = \Delta S^o_{CONF} + \Delta S^o_{SOLV}$

$\Delta S^o_{SOLV} = \Delta S^o_{TOTAL} - \Delta S^o_{CONF}$

= 600 – 1147
= - 547 J/mol-deg

iv) Calculate how much of this protein would be unfolded at 343 K (4 pts).

ΔG^o = -RT ln K_{EQ}

ΔG^o = ΔH – T ΔS

= 200000 – 343 x 600
= -5.8 kJ/mol

$K_{EQ} = e^{-\Delta G/RT}$

= $e^{5800/(8.3 \times 343)}$
=7.6

$F_U = K_{EQ}/(1+K_{EQ})$ = 7.6/8.6 = 0.88

9. (12 pts) Please do **one** of the following four choices. Please indicate your choice. The following equations may be useful, but a graphical solution is also acceptable.

$$pH = pK_A + \log\frac{[A^-]}{[HA]} \qquad R = 10^{pH-pKa}$$

$$f_{HA} = \frac{1}{(1+R)} \qquad\qquad f_{A^-} = \frac{R}{1+R}$$

Choice A (Buffer construction): You wish to make a buffer solution at pH 3.0 using phosphoric acid (H_3PO_4) as the starting material. How much NaOH do you have to add to 1L of solution to make a 1 molar solution of this buffer? You may assume that the pKa values of phosphate are 2, 7, and 12. (Hint: Assume phosphate is a monoprotic buffer with a pK_a=2).
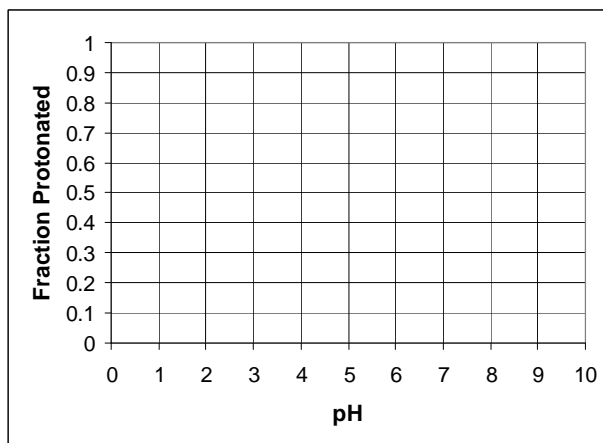
The pH is one unit above the pKa, so the fraction protonated is approximately 0.1. Therefore you would have to add 0.9 moles of base to reduce the amount of protonated acid to 0.1

Using the H&H equation: $R=10^{3-2}= 10$, $f_{HA} = 1/(11) = 0.091$, i.e., the graphical approximation gives a modest, 10% error.

Choice B (pH adjustment): A 1 M buffer solution of phosphate at pH = 3.0 is being used to buffer a reaction. The reaction that you are buffering absorbs protons, causing the pH to increase to 5.0. How much HCl do you have to add to restore the pH to 3.0? You may assume that the pKa values of phosphate are 2, 7, and 12. (Hint: Assume phosphate is a monoprotic buffer with a pK_a=2).

At pH 3.0, the fraction protonated is approximately 0.1. At pH 5.0 it will be essentially zero, so you would have to add 0.1 moles of acid to return the pH back to 3.0.

Using the H&H equation:

@pH 3: $R=10^{3-2}= 10$, $f_{HA} = 1/(11) = 0.091$

@pH 5: $R=10^{5-2} = 1000$, $f_{HA} = 1/1001 = .001$

Choice C (Charge calculation): What is the net charge at pH 9 for the amino acid that you selected for your answer in problem 1? Please show your work. (The structures are repeated here for your convenience.)
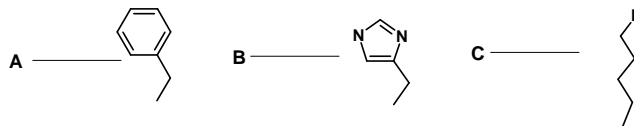
There are three ionizable groups. The carboxyl (pK_a=2), the histidine sidechain (pK_a=6), and the amino group (pK_a=9). Both the carboxy and the histidine are fully deprotonated at pH=9. The deprotonated – COOH has a charge of -1 and the deprotonated Histidine sidechain has a charge of zero.

The amino group is 50% protonated, so its average charge is +1/2. So the overall net charge is -1/2.

Choice D (Enzyme activity & pH): An enzyme has an Aspartic acid residue that is important for function. The enzyme activity at pH=4.0 is ~10%, at pH=5.0 it is 50%, and at pH=6.0 it is ~90%. What is the pKa of this group? Which form is active (protonated or deprotonated)? Justify your answers to both of these questions.

The pKa is 5.0 since the activity is at 50% at that pH.

The deprotonated form is the active form since the activity increases as the pH increases.