1. (20 pts) Acid-base, reactivity, and artistic properties of lysine.

One of the active site lysines (324) in the enzyme fumarase has a $pK_a$ of 7.1, surprisingly low.

(a; 3 pts) Complete the positively charged lysine side chain on the sketch below and write down the chemical equilibrium corresponding to its deprotonation.

(b; 3 pts) It turns out that the active site has a second lysine next to Lys324. The second one has a normal $pK_a$. How does this help explain the unusual $pK_a$ of Lys324?

The adjacent charge destabilizes the proton on Lys324. This makes it a stronger acid.

Score for the page: __________
(c; 5 pts) Use the Henderson-Hasselbach equation to calculate the ratio of deprotonated to protonated lysine at both pH 5.9 and at pH 8.1 for Lys324, with its $pK_a$ of 7.1.

\[
\text{pH} = pK_a + \log \left( \frac{[\text{Lys-}N\text{H}_2\text{]}_2}{[\text{Lys-}N\text{H}_3^+]_2} \right)
\]

\[
5.9 = 7.1 + 10^{-1.2} \quad +1
\]

\[
\frac{[\text{Lys-}N\text{H}_2\text{]}_2}{[\text{Lys-}N\text{H}_3^+]_2} = 0.063 \quad +1
\]

\[
8.1 = 7.1 + \log \left( \frac{[\text{Lys-}N\text{H}_2\text{]}_2}{[\text{Lys-}N\text{H}_3^+]_2} \right)
\]

\[
\frac{[\text{Lys-}N\text{H}_2\text{]}_2}{[\text{Lys-}N\text{H}_3^+]_2} = 10^{1.1} = 10 \quad +1
\]

(d; 5 pts) Because Lys324 has such a low $pK_a$, it is a much better nucleophile than free Lys. Draw the product of acetylating Lys324. Why is acetyl-lysine important in gene regulation?

![Diagram of acetylated lysine]

Acetylated lysine on histone proteins (in chromatin) is associated with transcriptional activation.

(e; 4 pts) Write down the name of a molecular visualization program and describe how to select and emphasize one residue using the program.

> Select (#, #)
> Spacebar
> +1 each or +2 for reasonable explanation

Score for the page______
2. **(20 pts) Rite of Passage:**

Draw the structure of the peptide Ile-Tyr-Met-Asp, including the correct stereochemistry at Cα's and all ionizable groups in their correct protonation states at pH 7. P-tyr = phosphotyrosine, which has $pK_a$'s of $-2$ and $-5.8$, so its charge at pH 7 is $-2$. If you need more space, the sequence is also on the last page.

3. **(25 pts) Bioinformatics:**

(a: 12 pts) Briefly describe the four steps in a typical bioinformatics/biochemistry "workflow" that a bench biochemist might perform in learning what she can about the likely structure and function of a protein sequence that she has just connected to a function of interest. Don’t forget the last and most important step!

1. Do a **BLAST** search to identify homologous sequences in the databases.
2. Evaluate the statistical significance of the match.
3. Perform **PSI-BLAST** or other search for family members.
4. Attempt to predict 3-D structure of your protein using any available structures of homologs (threading).
5. Formulate a hypothesis about the function of your protein.
6. Go into the lab and test the hypothesis!

Score for the page__________
(b; 13 pts) Contemplation of the BLOSUM matrix can provide much insight into protein and amino acid properties. Referring to the pictorial version of the top half of the substitution matrix, answer the questions below, whose numbers correspond to the indicated areas on the matrix:

1. Why are the scores for identical W, C, P, and H residues higher than the scores for other residues? (One answer for all three).

   These are all \textbf{vanishingly small} amino acids—matching is less likely to be accidental.

2. Why are I and V more similar to each other than either is to L, given that L and I are isomers of each other? In general, why do the bulky hydrophobic residues appear to substitute for each other quite readily?

   - I and V are \textit{β}-branched, so they fit similarly into sheet/helix structures, as opposed to \textit{α}-branched L.
   - Hydrophobic interactions are \textit{non-specific} so these amino acids are more interchangeable than e.g. H-bond groups.

Score for the page: ________
(3) Replacing E with K has a positive similarity score. Why is this initially surprising? Considering where the residues are likely to be located in the protein structure, explain why they do in fact often substitute for each other.

- E and K are oppositely charged - seen very different

3. Since they are both likely to be on the surface of the protein and hence solvated, they can both perform the same function of solubilization (or perhaps serve other functions).

(4) What are the special features of C, G, and P that make each of them different from all other amino acids (one feature each).

+1 - C makes disulfide bond
+1 - G is the most flexible
+1 - P is the only cyclic aa, the only one that can make cis peptide bonds

4. (35 pts) Secondary, tertiary, and miscellaneous structure:

(a) 8 pts In general, how do chaperones use the free energy available from ATP hydrolysis to improve the success rate of protein folding and avoid aggregation?

+2 - They bind to exposed hydrophobic surfaces.
+2 - ATP hydrolysis powers a conformational change that relays to unfolded protein
+2 - The protein then has a chance to refold on its own
+2 - The barrel cavity isolates the mis/unfolded protein from other copies of itself to prevent aggregation.

Score for the page: ________
(b; 9 pts) The two sequences below are known to be amphipathic. Which one is part of a beta sheet and which one is an alpha helix? Briefly explain your reasoning. For your convenience seven-pointed stars are sketched below in case you need them.

Sequence A: L E D K V E E L S S K N Y H L E N E V A R L
1 2 3 4 5 6 7 8 9 10 12 14 16 18 20 22

Sequence B: E I K N G I D L T L K A H M T F K I S F K W

(c; 4 pts) Under what conditions is an exothermic reaction thermodynamically favorable? Give an example of an exothermic ordering reaction.

\[ \Delta H < 0 \]
\[ \Delta S < 0 \]
\[ \Delta G = \Delta H - T \Delta S \]

\[ \Delta G > 0 \text{ at low } T, \text{ unfavorable at high } T \]
(d; 9 pts) Explain why molecule (i) below forms a micelle, molecule (ii) forms a bilayer, and molecule (iii) forms a globule. Briefly describe two biological functions for membranes.

(i) is an alkyl chain with a polar head group - triangular/lamellar pack into a sheet. The head groups don't face in!!

(ii) is a phospholipid with a rectangular/cylindrical shape - packs into a sheet - lipids classify head group vs. alkyl chain.

(iii) is much more hydrophobic - not amphipathic, so it excludes water completely.

1. Creation of concentration gradients for energy transduction, signaling
2. Communication with the outside world
3. Protection/preservation of the cell's contents

Score for the page: _______
(c: 5 pts) How does the steric zipper model provide a quite general and yet individualized failure mode for proteins?

- Many different short peptides can form steric zipper-clear that the model can explain many examples of protein misfolding.

- But the zipper is sequence-specific and there are many possible β-sheet topologies—therefore there is limited “cross talk” among proteins.

Most people did not know that this question referred to the β-sheet model presented on overheads.

<table>
<thead>
<tr>
<th>Page</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

Score for the page: 15