Biochemistry 110 – Midterm Exam Solutions
Fall 2016

• The FIRST EXAM QUESTION is to write the one-letter code, the three-letter code, and the structure of the twenty canonical amino acids. This section is closed book with a 1 hour time limit.

• The second section of the exam is open book and the time limit is 4 hours. For the “open book” portion of the exam, you may use the following materials:
  - Your Stryer ‘Biochemistry’ textbook
  - Your lecture notes and homework problem sets
  - Any material that has been posted on the Biochemistry 110 website

• You may have one break for any amount of time during the closed book portion of the exam. During this break you may not consult any Bi/Ch110 materials or talk to other students about the exam.

• Please have each problem of the exam on a separate page with your name on every page of the exam. You may have multiple pages for the same problem but you must have your name on every page.

• The due date is at the beginning of class (11 am) on Tuesday, November 1, 2016.

Section 1:
1. _____ /20

Section 2:
2. _____ /5
3. _____ /5
4. _____ /21
5. _____ /17
6. _____ /23
7. _____ /20
8. _____ /14
9. _____ /20
10. _____ /15
11. _____ /16
12. _____ /24

Total: _____/200
Section 1: Closed Book, 1-hour time limit

Problem 1: Amino Acids (20 points)

Draw the twenty canonical amino acids (encoded in biological proteins) as they would appear at pH = 7. Next to each amino acid, give its name and both its 3-letter and 1-letter identifications.

Amino acid side chains:

*note selenocysteine is not one of the 20 natural amino acids
Section 2: Open Book, 4-hour time limit

Problem 2: Protein Secondary Structure (5 points)

a) (3 pts) What are the most common secondary structures found in proteins and what are the differences between them? Please limit your answer to 4 sentences maximum.

Secondary structure are formally defined by the pattern of hydrogen bonds of the protein and are usually as alpha and beta sheets. Secondary structure does not describe the specific identity of amino acids in the protein and it doesn’t describe the atomic position in the three dimensional space. It basically gives information about the specific hydrogen bonds between amino acids. There are some other secondary structures such as 310 helix and π helix, however, they are not very common and are not characterized as most common and favorable hydrogen bonding patterns in natural proteins. Amino acids vary in their ability to form the various secondary structure elements. Proline and glycine are sometimes known as "helix breakers" because they disrupt the regularity of the α helical backbone conformation; however, both have unusual conformational abilities and are commonly found in turns. Amino acids that prefer to adopt helical conformations in proteins include methionine, alanine, leucine, glutamate and lysine ("MALEK" in amino-acid 1-letter codes); by contrast, the large aromatic residues (tryptophan, tyrosine and phenylalanine) and Cβ-branched amino acids (isoleucine, valine, and threonine) prefer to adopt β-strand conformations. However, these preferences are not strong enough to produce a reliable method of predicting secondary structure from sequence alone.

b) (2 pts) What secondary structure(s) are found in soluble proteins? Explain in 2 sentences maximum.

Both alpha-helices and beta-sheets are found in soluble proteins. Both structures are frequently found in soluble proteins unlike membrane proteins which favor alpha-helices because of their internal H-bond fulfillment.
Problem 3: DNA and RNA Processing (5 points)

a) (2pts) Define introns vs exons in DNA structure. Please limit your answer to 2 sentences maximum.

In most eukaryotic genes, coding regions (exons) are interrupted by noncoding regions (introns).

b) (3pts) Describe how RNA is typically processed during transcription. Please use a drawing in your explanation and limit your description to 3 sentences maximum.

During transcription, the entire gene is copied into a pre-mRNA, which includes exons and introns. During the process of RNA splicing, introns are removed and exons joined to form a contiguous coding sequence. This "mature" mRNA is ready for translation.

Figure 1: Pre-mRNA splicing.
Splicing of a pre-mRNA molecule occurs in several steps that are catalyzed by small nuclear ribonucleoproteins (snRNPs). After the U1 snRNP binds to the 5' splice site, the 5' end of the intron base pairs with the downstream branch sequence, forming a lariat. The 3' end of the exon is cut and joined to the branch site by a hydroxyl (OH) group at the 3' end of the exon that attacks the phosphodiester bond at the 3' splice site. As a result, the exons (L1 and L2) are covalently bound, and the lariat containing the intron is released.
Problem 4: DNA Structure and Organization (21 points)
For the multiple choice questions below please select the most appropriate answer for each question by writing the letter of the response you think is correct.

a) (3pts) Certain restriction enzymes produce cohesive (sticky) ends. This means that they:
A. cut in regions of high GC content, leaving ends that can form more hydrogen bonds than ends of high AT content.
B. make a staggered double-strand cut, leaving ends with a few nucleotides of single-stranded DNA protruding.
C. stick tightly to the ends of the DNA it has cut.
D. have all of the above characteristics
E. have none of the above characteristics.

B

b) (3pts) The Watson-Crick base pairing scheme for an A-T base pair includes:
A. a hydrogen bond between a keto oxygen and an extracyclic amino group.
B. a hydrogen bond between two ring nitrogen atoms.
C. an ionic bond between the positively charged adenine amino group and a negatively polarized keto group.
D. both A and B.
E. both B and C.

D

c) (3pts) In a Watson-Crick base pair for an A-T, how would the hydrogen bonds change if the adenine base were in its imine tautomer?
A. The extracyclic imino group would become a hydrogen bond acceptor.
B. The three hydrogen bonds to thymidine would break.
C. The methyl group of adenine would make hydrophobic contact with thymine
D. The two hydrogen bonds to thymidine would break.
E. The keto oxygen would become a hydrogen bond donor as a hydroxyl.
F. both A and D
G. both A and E

F.
d) (3pts) The fundamental repeating unit of organization in a eukaryotic chromosome is:
   A. the centrosome.
   B. the nucleosome.
   C. the lysosome.
   D. the microsome.
   E. none of the above.

   B

e) (3pts) In 1-4 sentences please describe the structural components and organization that create a nucleosome.

   A nucleosome is comprised of a **histone octamer** -- an eight protein complex found at the center of a nucleosome core particle. It consists of two copies of each of the four core histone proteins (H2A, H2B, H3 and H4). The octamer assembles when a tetramer, containing two copies of both H3 and H4, complexes with two H2A/H2B dimers.

f) (3pts) Describe one difference between the activity of DNA and RNA polymerase. Please limit your response to a maximum of 3 sentences.

   To initiate this reaction, DNA polymerases require a primer with a free 3′-hydroxyl group already base-paired to the template. They cannot start from scratch by adding nucleotides to a free single-stranded DNA template. RNA polymerase, in contrast, can initiate RNA synthesis without a primer.

g) (3pts) In one sentence, identify the most obvious structural difference between B-form (Watson-Crick) DNA and Z-form DNA.

   A-form has a right-handed helix; Z-form has a left-handed helix.
Problem 5: Cloning (17 points)

In a hypothetical scenario, after a campus lockdown and the regrettable ingestion of your emergency stock of ancient chandler food items, your roommate has developed a bizarre scale growth. You take samples of the ancient chandler plate (otherwise purple and unidentifiable) to your lab and you find that it contains a fungus that produces a protein (the ScalE protein) that stimulates scale growth. You construct a genomic DNA library in E. Coli with the hope of cloning the ScalE gene. You obtain DNA from the fungus, digest it with a restriction enzyme, and clone it into a vector.

a) (3pts) What features must be present on your plasmid that will allow you to use this as a cloning vector to make fungal genomic DNA library? Please use 1 sentence to describe these features.

Your vector would certainly need to have a unique restriction enzyme site (1pt), a selectable marker such as the ampicillin resistance gene (1pt), and a bacterial origin of replication (1pt). Other features may be required depending upon how you plan to use this library.

b) (4pts) You clone your digested genomic DNA into this vector. The E. coli (bacteria) cells that you will transform to create your library will have what phenotype prior to transformation? Please limit your response to a maximum of 2 sentences.

Prior to transformation, the E. coli cells that you will transform will be sensitive to antibiotic. This allows you to select for cells that obtained a plasmid.

c) (4pts) How do you distinguish bacterial cells that carry a recombinant vector from those that carry the original cloning vector? Please limit your response to a maximum of 3 sentences.

All cells with a vector, whether the original vector or a recombinant vector will have obtained the selectable marker. To distinguish bacterial cells that carry a recombinant vector from those that carry the original cloning vector, you would have to isolate the vector and determine its size. You could do this by restriction mapping.

d) (6 pts) You screen your library by hybridization with a probe and identify a recombinant vector that contains the complete ScalE gene. In the meantime, you have developed an antibody to the ScalE protein. You use cells carrying the recombinant vector that contains the complete ScalE gene to test how well this antibody reacts with the ScalE protein. You do the experiment and find that the antibody does not react with the cells containing the recombinant vector. Does
this result indicate that your antibody does not react to the ScalE protein? Please explain using a maximum of 4 sentences.

No. Screening with an antibody requires that the ScalE gene is expressed into protein. Your library used genomic fungal DNA and is contained in bacterial cells. Because of the difference in promoters and the presence of introns, the bacterial cells cannot express the ScalE protein. Thus you have no information about the ability of your antibody to bind.
Problem 6: Biological Membranes and Hydropathy (23 points)

Shown below is a table of hydropathies adapted from the hydropathy scale first derived by Kyte and Doolittle (Jol Mol. Bio. (1982) 157 105-132.)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>3-Letter Code</th>
<th>1-Letter Code</th>
<th>Molecular Weight</th>
<th>Hydropathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>Ala</td>
<td>A</td>
<td>89.09</td>
<td>1.8</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Cys</td>
<td>C</td>
<td>121.16</td>
<td>2.5</td>
</tr>
<tr>
<td>Aspartate</td>
<td>Asp</td>
<td>D</td>
<td>133.10</td>
<td>-3.5</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Glu</td>
<td>E</td>
<td>147.13</td>
<td>-3.5</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Phe</td>
<td>F</td>
<td>165.19</td>
<td>2.8</td>
</tr>
<tr>
<td>Glycine</td>
<td>Gly</td>
<td>G</td>
<td>75.07</td>
<td>-0.4</td>
</tr>
<tr>
<td>Histidine</td>
<td>His</td>
<td>H</td>
<td>155.16</td>
<td>-3.2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Ile</td>
<td>I</td>
<td>131.18</td>
<td>4.5</td>
</tr>
<tr>
<td>Lysine</td>
<td>Lys</td>
<td>K</td>
<td>146.19</td>
<td>-3.9</td>
</tr>
<tr>
<td>Leucine</td>
<td>Leu</td>
<td>L</td>
<td>131.18</td>
<td>3.8</td>
</tr>
<tr>
<td>Methionine</td>
<td>Met</td>
<td>M</td>
<td>149.21</td>
<td>1.9</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Asn</td>
<td>N</td>
<td>132.12</td>
<td>-3.5</td>
</tr>
<tr>
<td>Proline</td>
<td>Pro</td>
<td>P</td>
<td>115.13</td>
<td>-1.6</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Gln</td>
<td>Q</td>
<td>146.15</td>
<td>-3.5</td>
</tr>
<tr>
<td>Arginine</td>
<td>Arg</td>
<td>R</td>
<td>174.20</td>
<td>-4.5</td>
</tr>
<tr>
<td>Serine</td>
<td>Ser</td>
<td>S</td>
<td>105.09</td>
<td>-0.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>The</td>
<td>T</td>
<td>119.12</td>
<td>-0.7</td>
</tr>
<tr>
<td>Valine</td>
<td>Val</td>
<td>V</td>
<td>117.15</td>
<td>4.2</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Trp</td>
<td>W</td>
<td>204.23</td>
<td>-0.9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyr</td>
<td>Y</td>
<td>181.19</td>
<td>-1.3</td>
</tr>
</tbody>
</table>

a) (3pts) What does the hydropathy value indicate with regards to the amino acids?
As the value for hydropathy increases, the amino acids become more hydrophobic. This means that these residues are more likely to reside in the hydrophobic regions of the lipid bilayer. (3 points)
Consider the following sequence:
MTNREHAFIVFCWKRPYTLVIAGASDKRQHT

b) (10 pts) Sketch a hydropathy plot for this sequence.
10 points total; 3 points for labeling axes correctly and 7 points for graphing the points and coming up with the appropriate trend. Since there are over 30 amino acids here, I think it’s alright if each point is not meticulously plotted. I plotted this one using Expasy, which I think does some sort of scaling; however, the trend should be the same.

c) (5 pts) Based on your plot, what kind of secondary structure would you expect, and where would you expect this portion of the sequence to reside? Why?
I would expect this to be an alpha helix that is part of a transmembrane protein. There are distinct regions of hydrophobic and hydrophilic residues within the sequence, which would indicate alpha helices spanning the membrane, then looping back. (5 points)

d) (5 pts) From your analysis, make a rough sketch of what this protein’s structure would look like. Include the membrane.
5 points total; 3 for drawing out the alpha helices, and 2 points for drawing the membrane appropriately.

Note that proteins should be 20 amino acids long to span the membrane, so we also accepted this answer (i.e. that the helices can’t fully cross the membrane since they are not long enough).
Problem 7: The Hydrophobic Effect (20 points)

a) (10 pts) According to the hydrophobic effect, hydrophobic molecules have a tendency to cluster and aggregate with themselves. However, from an entropic standpoint, it would seem that the self-assembly of phospholipids to form bilayers results in a more ordered system. However, the formation of bilayers is entropically favorable. Explain this paradox.

Since the hydrophobic effect is a thermodynamically driven process, entropy plays a key role in its effect. When hydrophobic molecules are put into a solution of water, they can either randomly move around individually or clump together. In the first case, lots of water is needed to individually solvate the molecules, which forms ordered cages around the molecules of a solution (thus decreasing the entropy). On the other hand, with a clustered group of hydrophobic molecules, less water is needed to solvate the clump, which results in less ordering of water molecules. This means that the observed tendency for the hydrophobic molecules to cluster is the most entropically favorable state. (10 points)

b) (10 pts) Draw a diagram that demonstrates the hydrophobic effect, starting with three phospholipids in a solution of water. 10 points; 5 for drawing the phospholipids and water accurately, and 5 for demonstrating the decreased ordering of water molecules in the aggregated state.
Problem 8: Protein Folding (16 points)

a. (5 points) If a 50 amino acid polypeptide were to sample all of its possible conformations in order to fold, how long would this process take? Assume that each amino acid residue can have three different conformations and it takes one picosecond ($10^{-12}$) to convert between structures.

b. (5 points) Describe how proteins can fold in time scales less than you calculated in (a).

c. (4 points) In the research paper from which the figure below originates, the authors used hydrogen-deuterium exchange and mass spectrometry to determine folding intermediates of the protein ribonuclease H.

i. (2 points) In the experiment, the authors unfold ribonuclease H in the presence of D$_2$O and then refold the protein in the presence of H$_2$O. If an amino acid residue shows a high D:H ratio, what does this imply? What about a low D:H ratio?

ii. (2 points) The figure below shows the results of this deuterium-hydrogen exchange experiment over time, with the bottom panel being the native protein. The y-axes show the “protection factor” of deuterium, with 1 showing “high” protection (ie a high D:H ratio) and 0 showing “low” protection (ie a low D:H ratio). “In transition” means that there is too much of a mix of D and H, and residue-by-residue data cannot be resolved. A cartoon of the protein structure is shown above the data. What is the secondary structure elements in the folding intermediates of ribonuclease H? Please explain your answer.
Problem 8: Protein Folding (14 points)

a. (5 points) If a 50 amino acid polypeptide were to sample all of its possible conformations in order to fold, how long would this process take? Assume that each amino acid residue can have three different conformations and it takes one picosecond ($10^{-12}$) to convert between structures.

Number of structures: $3^{50} = \sim 7 \times 10^{23}$

Time it takes to sample: $7 \times 10^{23} \times 10^{-12} \text{ s} = 7 \times 10^{11} \text{ s} = \sim 2 \times 10^{8} \text{ minutes} = \sim 2 \times 10^{8} \text{ hours} = \sim 8 \times 10^{6} \text{ days} = \sim 22,000 \text{ years}$

b. (5 points) Describe how proteins can fold in time scales less than you calculated in (a).

In the model of the progressive stabilization of intermediates, the correct or partially correct structure are generally retained while the incorrect structures can then sample a new conformation, greatly reducing the number of conformations that the protein must sample before it finds the lowest energy structure. This allows for transient folding intermediates to form, giving local regions strong preferences for a particular structure that then in turn influence the stabilities of nearby regions. This is also called the “nucleation-condensation model.” It can also be visualized as a protein folding funnel, where as you go down in energy, semistable intermediates form that help (or hinder) native protein formation before the secondary structures collapse to initiate folding.

c. (6 points) In the research paper from which the figure below originates, the authors used hydrogen-deuterium exchange and mass spectrometry to determine folding intermediates of the protein ribonuclease H.

i. (2 points) In the experiment, the authors unfold ribonuclease H in the presence of D$_2$O and then refold the protein in the presence of H$_2$O. If an amino acid residue shows a high D:H ratio, what does this imply? What about a low D:H ratio?

If a residue is in a stably folded region of the protein, then its deuterium/hydrogen are not accessible for exchange. Therefore, a high D:H ratio implies that this residue is in a folded region of the protein, and a low D:H ratio implies that this residue is not in a folded region of the protein.

ii. (4 points) The figure below shows the results of this deuterium-hydrogen exchange experiment over time, with the bottom panel being the native protein. The y-axes show the “protection factor” of deuterium, with 1 showing “high” protection (ie a high D:H ratio) and 0 showing “low” protection (ie a low D:H ratio). “In transition” means that there is too much of a mix of D and H, and residue-by-residue data cannot be resolved. A cartoon of the protein structure is shown above the data. What is the secondary structure elements in the folding intermediates of ribonuclease H? Please explain your answer.
Helix A (and strand 4) $\rightarrow$ (strand 4) $\rightarrow$ helix B, helix C, helix D, strand 5 $\rightarrow$ helix E, strand 1, strand 2, strand 3
(it’s ok if strand 4 is bundled with helix A or in its own separate folding intermediate)

A high “protection factor” means that these residues are found in folded regions of the proteins. The first highly protected, and therefore folded, regions are helix A and strand 4. Helix B, helix C, helix D, and strand 5 appear next in time to be protected, meaning they form another folding intermediate. Finally, in the folded protein, helix E, strand 1, strand 2, and strand 3 collapse to form a folded structure.

Figure from: Hu et al (2013) *Proc Natl Acad Sci USA* **110**: 7684-7689.
Problem 9: Chaperonins: GroEL/ES (20 points)

a) (4 points) Reconcile these seemingly contradictory statements: a protein’s structure is determined only by its amino acid sequence AND chaperonins are necessary for some proteins to fold correctly. Please limit your answer to a maximum of 4 sentences.

b) (8 points) Explain how chaperonins affect the following:
   i. Kinetics of folding (4pts)
   ii. Thermodynamics of folding (4pts)

c) (4 points) Rationalize the following observation: the surface/top of the central cavity in GroEL is hydrophobic, but the central cavity itself is hydrophilic. Please limit your answer to a maximum of 3 sentences.

d) (4 points) What would you expect to happen if GroEL is mutated so that it cannot hydrolyze ATP? Why? Please limit your answer to a maximum of 3 sentences.

Problem 9: Chaperonins: GroEL/ES (20 points)

a. (4 points) Reconcile these seemingly contradictory statements: a protein’s structure is determined only by its amino acid sequence AND chaperonins are necessary for some proteins to fold correctly.

   The lowest-energy structure of a protein is determined by its amino acid sequence. However, sometimes while proteins are folding, they reach kinetically-trapped products that are not the lowest energy but have too high an energy barrier to overcome to reach the lowest energy state. In this case, a chaperonin is needed to ensure that a protein does not form these trapped products or form aggregates, allowing it to reach the native structure dictated by the sequence. Chaperonins do not actively remodel the protein.

b. (8 points) Explain how chaperonins affect the following:
   i. Kinetics of folding (4pts)

      Chaperonins speed up the kinetics of folding by preventing their falling into kinetic traps, such as aggregates, and reducing the activation energies to get out of incorrectly folded states.

   ii. Thermodynamics of folding (4pts)

      Chaperonins have no effect on the thermodynamics of folding (ie the lowest-energy native state of the protein).
c. (4 points) Rationalize the following observation: the surface/top of the central cavity in GroEL is hydrophobic, but the central cavity itself is hydrophilic.

The surface of the GroEL cavity is hydrophobic so that misfolded proteins which have their hydrophobic cores exposed will bind. The central cavity is hydrophilic to promote proper folding for an aqueous environment. In this hydrophilic environment, the hydrophobic core of the protein has nothing to interact with except itself.

d. (4 points) What would you expect to happen if GroEL is mutated so that it cannot hydrolyze ATP? Why?

The hydrolysis of ATP causes the substrate/client unfolded protein and GroES to be released from GroEL. Therefore, a GroEL that cannot hydrolyze ATP will have unfolded protein irreversibly bound.
Problem 10: Selectivity of Membranes (15 points)

a) (6pts) The curves below represent one of the following possibilities: (1) transport with a transporter protein and no competition, (2) simple diffusion, or (3) transport with a transporter protein and competition. Explain which curve correlates to which of these scenarios and why. Please limit your answer to 1 sentence of explanation per curve.

A = 2; linear, therefore diffusion because it is not limited by protein concentration (2pt)
B = 1; reaches a plateau because of transporter concentration, not competition because it reaches saturation sooner (2pt)
C = 3; reaches saturation later than B, therefore has competition (2pt)

b) (3 pts) Why might neural cells need to move ions against their concentration gradient? Please limit your response to a maximum of 3 sentences.

To produce a membrane potential capable of generating electrical signals. To distribute ions to where they are needed for functions in proteins.

c) (6 pts) Would the following molecules be transported across the membrane through diffusion or would they require a transporter and why? (1) Zn\(^{2+}\) (2) ATP (3) ethanol. Please limit your answer to 1 sentence per explanation for each molecule.

1) Needs a transporter because it is charged (2pts)
2) Needs a transporter because it is charged (2pts)
3) Partially permeable by diffusion because it is small and uncharged but polar; therefore doesn’t necessarily need a transporter (2 pts)
Problem 11: Mechanisms of transporters (16 points)

a) (3pts) How might an active transporter discriminate between the following ions: Ca\(^{2+}\), Mg\(^{2+}\), K\(^+\)? Please limit your response to 3 sentences maximum.

Charge (1+ vs 2+) and size of ions

b) (3pts) Imagine that SERCA has its Asp 351 mutated to a leucine. How would this mutation affect calcium transport in a cell and why? Please limit your response to 3 sentences.

The phosphate would no longer bind to the aspartate site, which would prevent ATP hydrolysis. Calcium would become trapped in the transporter and would not be able to go to the cytoplasm from the lumen. This is because leucine can no longer accommodate the phosphate group as aspartic acid could.

c) (10 pts) In class we discussed various transporter molecules and mechanisms. On transporter that we did not focus on specifically is the sodium/calcium exchanger. This transporter pumps Na\(^+\) into cells and Ca\(^{2+}\) out of cells. For every 3 Na\(^+\) that come into the cell, 1 Ca\(^{2+}\) goes out.

i. What type of transport does this process represent? (2 pts)
   Secondary active transport or facilitated passive transport (both answers accepted as well as antiporter).

ii. Using what you have learned about other transporters, sketch this transporter in the membrane indicating the flow of ions. Make sure to label the inside and outside of the cell. (2pts)

iii. The sodium/calcium exchanger is implicated in the cardiac electrical conduction abnormality known as delayed polarization. It is thought that intracellular accumulation of Ca\(^{2+}\) causes the activation of the sodium/calcium exchanger. Why might this activation lead to a cardiac arrhythmia? (2pts)
   Brief influx of positive charge (3 Na\(^+\) in, 1 Ca\(^{2+}\) out) causes cellular depolarization

iv. Given a membrane potential of -80 mV, a temperature of 37\(^\circ\) Celsius, and a concentration of Ca\(^{2+}\) that is 2 mM outside of the cell and 10\(^{-8}\) M inside the cell, a Na\(^+\) concentration of 145 mM outside of the cell and 15 mM inside of the cell, what is the free energy of transport to move Ca\(^{2+}\) out of the cell? Where might this energy come from in this process if ATP is not required? (4 pts)
\[ \Delta G = RT \ln \left( \frac{c_1}{c_2} \right) + ZF \Delta V \]

Celsius to Kelvin: 37 to 310K
\[
\Delta G = (2 \text{ kcal/mol*K})(310K) \ln(2*10^{-8} \text{ M}/10^{-8} \text{ M}) + (+2)(23,062\text{kcal/V})(-0.080\text{V})
\]
\[= 3878 \text{ or } 3.9 \text{ kcal/mol} \]

This energy comes from Na+ moving down it’s concentration gradient:
3 Na+ go from outside to inside
\[
\Delta G = (2 \text{ kcal/mol*K})(310K) \ln(15 \text{ mM}/145\text{mM}) + (3)(+1)(23,062\text{kcal/V})(-0.080\text{V})
\]
\[= -6941 \text{ or } -6.9 \text{ kcal/mol} \]

Did not grade for numerical answers on this problem. Only graded for setup of the equation since it was not clear that students could use a calculator.
Problem 12: Enzymatic Catalysis (24 points)

For the data acquired for the enzyme shown above:

a) Identify the pKa for each of the two-steps (shown by the two solid black lines) (4 points).

The pKa for each step is ~ 6.8.

b) What can you conclude about the catalytic site both in terms of: the most likely amino acid and the class of enzymes this belongs to (6 points)? Please explain in 3 sentences maximum.

This most likely corresponds to the imidazole group on Histidine. This implies that there is a common intermediate in the reaction mechanism. This is consistent with the family of enzymes that contain a catalytic triad.

c) Draw a generalized base catalytic mechanism for this common class of enzymes (6 points).
d) What are two criteria for the identity of the active residue and general acid/base catalysis (8 points)? Use 1 sentence to describe each criterion.

Possible answers:
1. Mutation of the candidate residue abolishes pH dependence of the reaction
2. Reaction of substrates with better nucleophile or leaving group is less dependent on the general acid/base
3. pKa of the candidate residue is the same as the pKa observed in the pH-rate profiles

Accepted a wide range of answers for this problem since it was a broad question.