

SSSS Protein Modeling ANSWER KEY

By: Clements HS

Instructions to participants:

This exam is divided into 4 sections. You will have 45 minutes to complete this test. This test is completely free-response to better test your understanding of these concepts. Due to this, partial credit will be awarded for questions with point values greater than 1.

Section 1: Biochemistry(40%) - 25 points

- Covers protein structure and folding
- Biological principles of proteins
- Nucleic Acids

Section 2: CRISPR(20%) - 12 points

- CRISPR in bacterial immunity
- CRISPR-cas9 engineering
- Types of CRISPR

Section 3: Cytidine Deaminase (15%) - 10 points

- Cytidine Deaminase function

Section 4: Jmol Exploration(25%) - 15 points

Part 1: Biochemistry

1. How do proteins maximize the strength of their hydrophobic interactions? (2 pts)

1 pt: Hydrophobic interactions b/w nonpolar groups

1 pt: Minimizing interactions with water.

2. What two amino acids don't form alpha helices easily and why? (1 pt)

0.5 pt: Proline and Glycine

0.5 pt: proline - too rigid and no rotation, glycine - too small and flexible

3. How many amino acids residues are there per turn of an alpha helix? How many angstroms in width is this? (1 pts)

0.5 pt: 3.6

0.5 pt: 5.4 A

4. How does hydrogen bonding in an alpha helix work? Draw a picture or explain in words. (1 pt)

Hydrogen bonding between every 4th amino acid in order to coil to form a tight helix.

5. What amino acid has the highest tendency to form a helix? (1 pt)

Alanine

6. Which 2 amino acids are solely ketogenic? (1 pts)

1 pt: Leucine and Lysine

7. What types of amino acids are found in the middle of Beta-sheets? (1 pt)

1 pt: Large aromatic AA's and Beta-branched amino acids

1 pt: mentioning 3 of Tyrosine, Phenylalanine, tryptophan, threonine, valine, isoleucine

8. What is the relationship between K_a and K_d ? What is the relationship between K_d and the affinity of ligands for a protein? (2 pts)

1 pt: reciprocal of K_a

1 pt: Lower value means higher affinity of a ligand for a protein (and vice versa)

9. How do enzymes catalyze reactions effectively? (2 points)

1 pt: orienting to minimize entropy

1 pt: desolvation of water shell

1 pt: induced fit/functional group arrangement

Maximum of 2 points

10. Derive the Michaelis-Menten equation. Hint - use the steady-state assumption as a starting point. (3 points)

0.5 pt $E + S \rightarrow [ES] \rightarrow E + P$;

0.5 pt show associated rate constant(k_1 from $E + S \rightarrow [ES]$ and k_2 from $[ES] \rightarrow E + P$).

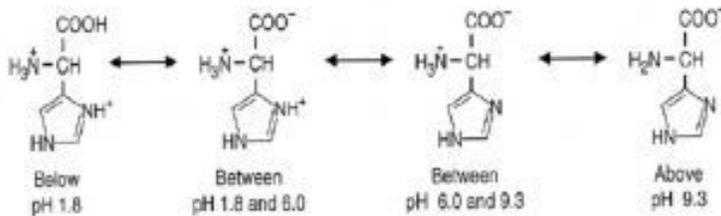
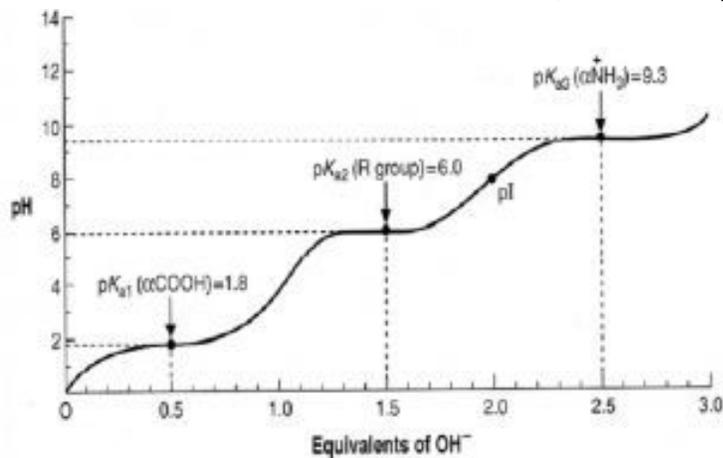
0.5 pt $V_0 = k_2[ES]$

0.5 pt Rate of ES formation = Rate of ES breakdown, let us define $[E_t]$ as total enzyme so $k_1([E_t] - [ES])[S] = k_{-1}[ES] + k_2[ES]$.

0.5 pt Defining $(k_2+k_{-1})/k_1$ as K_m and solving for $[ES]$, we get $[ES] = ([E_t][S]) / ([S] + K_m)$.

0.5 pt Using $V_0 = k_2[ES]$, we get $V_0 = (k_2[E_t][S]) / (K_m + [S])$ and assuming $V_{max} = k_2[E_t]$, we get $V_0 = (V_{max}[S]) / (K_m + [S])$

11. Draw a titration curve for histidine. Designate the pK_1 , pK_2 , pK_3 , and pI . (3 pts)



12. RNA and DNA are two important nucleic acids in eukaryotes. However, they differ in a considerable number of ways.

a) Identify how the sugar ribose differs from deoxyribose. (1 pt)

1 pt: Ribose has an additional 2' OH(hydroxyl) group that deoxyribose lacks.

b) What are the implications of this difference in structure with regards to stability of both of these molecules? (2 pts)

1 pt: Any answer that talks about self-catalyzed RNA hydrolysis cleaving a phosphodiester bond in the backbone

1 pt: Any answer that mentions this alkaline hydrolysis causing lower stability of RNA compared to DNA.

c) Describe the reaction mechanism by which the process in (b) occurs. If you would like you can draw a picture or you may describe this process in words. (2 pts)

2 pts: deprotonated 2' OH acts as a nucleophile that attacks the phosphorus molecule in the PO₄³⁻ which cleaves the phosphodiester bond that holds the sugar-phosphate backbone.

1 pt: alkaline conditions/deprotonation

1 pt: correctly identifying nucleophile + attack

d) What amino acids preferentially bind to DNA and what structures are they commonly found in? (2 pts)

1 pt: Arginine and Lysine(no credit for histidine)

1 pt: Histones

Part 2: CRISPR

1. Where is CRISPR-CAS9 naturally found and what is its purpose? (1 pt)

Bacteria/archaea(only need one) 0.5pt ; adaptive immunity defense mechanism against bacteriophages 0.5pt.

2.What is the PAM sequence and why is it important? (1 pt)

A few nucleotides downstream of the cut site, protospacer adjacent motif; No cleave can be made if sequence is absent.

3. What are the two possible repair pathways that might be activated after Cas9 cleaves the DNA? (2 pts)

1 pt. NHEJ

1 pt. HDR

4. What is the signature gene of the three most important types of CRISPR- Cas systems? (1.5 pts)

0.5 pt. Type 1 CRISPR-CAS3

0.5 pt. Type 2 CRISPR-CAS9

0.5 pt. Type 3 CRISPR-Cas10

5. Name and explain the mechanism behind two other gene editing techniques other than CRISPR(3 points)

0.75 pt. Zinc Finger Nuclease(ZNF)

0.75 pt. TALENs

0.75 pt. Reasonable explanation for ZNF(ZF DNA binding domain, Half Fok1 endonuclease, Fok1 dimerization)

0.75 pt. Reasonable explanation for TALENs(Similar to ZNF except nucleotide recognize rather than codons)

6. What are the two nucleases involved in DNA Repair? (1pt)

1pt. RuvC and HNH

No partial

7. What is AcrII-A4 and what is its function in regards to CRISPR and genome editing? (2.5 pts)

It is an Anti-Crispr protein that inhibits genome editing by preventing the binding of CRISPR protein complexes with the DNA. (1.25)

This beautifully illustrates that of the red queen hypothesis as phages evolve to fight the anti-viral mechanisms of bacteria. (1.25) has to at least mention "phages"

Part 3: Cytidine Deaminase

1. How does APOBEC3G cause a 5-methylcytosine to thymine mutation? (1 pt)

1pt. Removing an amino group from 5-methylcytosine(deamination)

2. Is the APOBEC3 family part of the adaptive or innate immune system? What is their specific role? (2 pt)

1 pt: innate

1 pt: Restricting retroviruses and mobile genetic elements like retrotransposons and transposons.

3. What is the role of APOBEC1? (1 pt)

1 pt: Deaminates apolipoprotein B messenger RNA to generate a premature stop codon.

4. What compounds are formed by the deaminase of cytidine/deoxycytidine?(1 pt)

0.33 pt: Uridine

0.33 pt: Deoxyuridine

0.33 pt: Ammonia/NH₃

5. What is the function of the catalytic zinc section of the APOBEC3 protein? (2 pt)

2 pt: Coordinates the water which acts the proton source for the amino group and N3 atom of cytosine

6. Targeting the APOBEC3A and APOBEC3B offer promising treatments for aggressive cancers. Out of those two proteins, which one is able to cause mutations at a higher rate? What are two plausible ways to treat cancers in regards to APOBEC3A and APOBEC3B expression?(3pt.)

1pt: APOBEC3A mutates genome at a higher rate

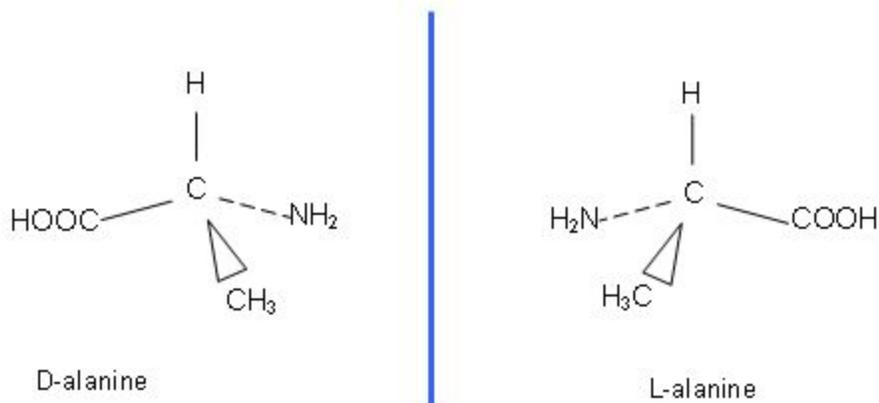
1pt: decrease expression for these proteins to decrease mutations

1pt: increase expression significantly to hopefully induce lethal mutations in tumor dna

Part 4: Jmol Exploration

Directions: Use Jmol to explore the crystal structure of nanobody D3-L11 mutant Y102A in complex with hen egg-white lysozyme (form II) [6jb5.pdb]

- The following questions refer to the entire structure [3 pts]
 - How many hydrophobic atoms are in this structure? 913 (0.5 pts)
 - How many polar atoms are in this structure? 1107 (0.5 pts)
 - How many atoms are selected when the structure is restricted to water molecules? 310 (0.5 pts)
 - How many chains are present in the structure? 2 (0.5 pts)
 - How many atoms are selected when the structure is restricted to nucleic acids? 0 (0.5 pts)
 - How many struts are in the structure? 39 (0.5 pts)
- The following questions refer to residue 35 of chain A [1.5 pts]
 - What is the one letter abbreviation of this amino acid? T (0.5 pts)
 - What is the chemical formula of this amino acid? $C_4H_9NO_3$ (0.5 pts)
 - How many chiral centers does this amino acid have? 2 (0.5 pts)
- The following questions refer to residue 23 of chain A [2 pts]
 - What is the full name of this amino acid? Alanine (0.5 pts)
 - Is the R-group of this amino acid aromatic or aliphatic? Aliphatic (0.5 pts)
 - Draw and label the two isomers of this amino acid (1 pt)



- The following questions refer to residue 100 of chain A [1 pt]
 - What is the full name of this amino acid? Arginine (0.5 pts)
 - True or False: The positional variability of atom #787 is greater than that of atom #777. True (0.5 pts)
- The following questions refer to chain B [2.5 pts]
 - How many hydrogen bonds are in chain B? 80 (0.5 pts)
 - How many atoms are in sheets? 69 (0.5 pts)

- c. How many 310 helices are present? 4 (0.5 pts)
 - d. What ligands are present? List both the abbreviated ID and the name of the ligands. GOL (glycerol), CL (chloride ion) (1 pt)
6. What is the default color for sodium? Purple (1 pt)
 7. What is measured by an atom's "temperature factor"? What command would be used to color a structure's atoms based on their "temperature factor"? Positional variability; color temperature (2 pts)
 8. What do struts represent? Struts are where structural supports may need to be placed to produce a solid and stable physical model for 3D printing (1 pt)
 9. What are disulfide bonds referred to in Jmol? ssbonds (1 pt)