Protein Modeling - Division C Exam

University of Texas-Austin Invitational

October 26, 2019

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Team Number: _____

Team Name: _____

Total Points: _____/160 **Rank:** _____

Instructions and Clarifications:

- You have **50** minutes to finish this exam and the computer exploration of protein structure. This exam is for **Part I** of the answer sheet. This exam accounts for **30%** of the event score.
- Each **participant** may bring **one** 8.5" x 11" sheet of paper that may be in a sheet protector or laminated that contain information without any annotations or labels affixed along with writing utensils for each participant.
- You **may** not write on this exam. Only the **answer sheet** will be graded.
- Write your team number on every page of the answer sheet.
- Tiebreakers are labeled as **TB#**. There are **five** tiebreakers in this exam.
- If you have any questions or comments about this exam, feel free to email me at velasco.scienceolympiad@gmail.com. **Happy testing!**

I. General Biochemistry of Macromolecules

Multiple Choice Directions: Choose the most appropriate answer for each question below. Each question is worth **one** point. **(10)**

1. Which of the following statements about DNA structure is correct?
    a. DNA is usually in single strand form.
    b. A nucleobase in DNA holds the chain together.
    c. The sugar in DNA contains six carbons.
    d. The sugar in DNA is called 2-deoxyribose.
    e. All of the above

2. What bases are present in DNA?
    a. Adenine
    b. Cytosine
    c. Guanine
    d. Thymine
    e. All of the above

3. Which of the following statements about nucleobase classification is correct?
    a. Adenine and cytosine are purines.
    b. Adenine and guanine are purines.
    c. Cytosine and adenine are pyrimidines.
    d. Cytosine and thymine are pyrimidines.
    e. All of the above

4. Which of the following is a noncanonical base that can be found in DNA?
    a. N6-carbamoyl-methyladenine
    b. 7-Methylguanine
    c. 5-Formylcytosine
    d. Base J
    e. All of the above

5. Which of the following statements about base pairing is correct?
    a. Purines form ionic bonds to pyrimidines.
    b. A Watson-Crick base pair is the arrangement of two nucleotides binding together in the double helix.
    c. Hydrogen bonds are not covalent bonds.
    d. High temperature cannot pull DNA strands apart.
    e. All of the above

6. Which of the following statements about nucleotides is correct?
    a. DNA with a lower GC-content is more stable than DNA with a higher GC-content.
    b. DNA with a higher GC-content is more stable than DNA with a lower GC-content.
    c. GC-content does not contribute to the stability of a DNA helix.
    d. GC forms two bonds, whereas AC forms three bonds.
    e. All of the above

7. Which of the following correctly describes the structure of RNA?
    a. It is usually found in humans as a single stranded linear polymer.
    b. A nucleoside is connected to a base through the 1'C.
    c. A nucleotide is connected to the 5'C of the sugar.
    d. There is intrastrand pairing of bases in the secondary structure of RNA.
    e. All of the above

8. What ion stabilizes RNA structure when the structure has irregular hydrogen bond pairings?
    a. Ca^{2+}
    b. Na^{+}
    c. K^{+}
    d. Mg^{2+}
    e. All of the above

9. What is the main reason why RNA is not as stable as DNA?
    a. Because of the extra -OH group attached to 2' Carbon
    b. Because uracil is less stable than thymine
    c. Because it is single stranded, while DNA is double stranded
    d. Because of the hairpin loops that form in RNA
    e. All of the above

10. Because of the tertiary structure of this RNA type, it can bind to mRNA and help it degrade. What type of RNA is being discussed?
    a. miRNA
    b. snRNA
    c. RNAi
    d. siRNA
    e. All of the above

Short Answer Directions: Answer the short answer questions below in the most concise way possible. The point values are indicated by the number in the parentheses. **(30)**

1. Where do most of the net change in free energy that occurs when weak interactions (e.g. Van der Waal forces) are formed within a protein come from? (2)

2. Why is the peptide C-N bond shorter than the C-N bond in a simple amine? (2)

3. Why are peptide C-N bonds unable to rotate freely? (1)

4. There is a small electric dipole established by oxygen and nitrogen in a protein. Which element has a partial negative charge? Which element has a partial positive charge? (2) (2)

5. Why would a polypeptide with a long block of Glu residues not form an alpha helix at pH 7.0? (3)

6. Why is proline rarely involved in the formation of an alpha helix? (4)

7. What is the most common way of creating disulfide bonds? (2)

8. The rotation of protein chain can be described as the rotation of peptide bond planes relative to each other. List and describe the three torsion angles involved in protein formation. **TB#1** (6)

9. In the 1930s, Linus Pauling and Robert Corey studied the bond distances and angles in dipeptides. What did they discover was the length of the carbon-nitrogen peptide bond, carbon-nitrogen single bond, and carbon-nitrogen double bond in nanometers? (3)

10. Which is more stable: the trans isomer or cis isomer? Explain. (5)

Diagram-based questions: Use the following diagrams to help you answer the given questions. The point values will be addressed in each question. **(19)**

1. Use **Figure 1.1** in the image packet to answer the following questions. **Figure 1.1** refers to an amino acid that offers unique properties when it comes to protein folding. (9) **TB#2**

    A. What is the amino acid shown? (1)
    B. Why is this amino acid unique in terms of its chirality? (2)
    C. True or False: This amino acid can only fit into hydrophilic environments due to its minimal side chain of one hydrogen atom. (1)
    D. At a low pH, at what pK_a can this molecule be protonated? (2)
    E. At a high pH, at what pK_a does this molecule lose a proton? (2)
    F. Is this amino acid conformationally flexible or unfiexile? (1)

2. Use **Figure 1.2** in the image packet to answer the following questions. The questions refer to characteristics of beta sheets in general, parallel and antiparallel beta sheets. (10)

    A. Where are the hydrogen bonds located in beta sheets? (1)
    B. What is the orientation of the R-groups of neighboring residues in a beta-strand? (1)
    C. What is the axial distance between adjacent residues of a beta strand in Angstroms? (2)
    D. In terms of direction they run, how do parallel beta sheets run in contrast with antiparallel sheets? (2)
    E. What do the dashed lines in the diagram represent? (1)
    F. In what type of protein are beta-sheets most common? (1)
    G. In the Pauling-Corey model, beta-sheets are planar. However, what is the orientation of most beta-sheets in globular proteins in X-ray structures? (2)

II. CRISPR-Cas Systems and Cytidine Deaminase

Multiple Choice Directions: Choose the most appropriate answer for each question below. Each question is worth **one** point. **(15)**

1. Which of the following is a signature gene for type II CRISPR-Cas system?
    a. Cas2
    b. Cas3
    c. Cas7
    d. Cas9

2. In what defense stage does Cas9 recognize the PAM?
    a. Adaptation
    b. CRISPR processing
    c. Interference
    d. None of the above

3. In what stage is the crRNA-foregin nucleic acid complex cleaved?
    a. Adaptation
    b. CRISPR processing
    c. Interference
    d. None of the above

4. In what stage of interference involves DNA cleavage interfering with viral replication, providing immunity to the host?
    a. 1
    b. 2
    c. 3
    d. None of the above

5. In what stage of interference are protospacers and protospacer-associated motifs acquired at the leader end of a CRISPR array in the host DNA?
    a. 1
    b. 2
    c. 3
    d. None of the above

6. Which of the following statements about CRISPR and its role in immunity is correct?
    a. It allows bacteria and archaea to find bacteriophages.
    b. It is not a self-programmable restriction enzyme.
    c. The palindromic repeats of CRISPR loci occur at irregular intervals.
    d. None of the above

7. Which of the following statements about interference of transcription by dCas9 is correct?
    a. The catalytic residues of the RuvC and HNH domain are mutated to lysine in order to use this enzyme to repress different genomic loci.
    b. The high molarity urea cannot fully dissociate from the dCas9 RNA-protein complex from the dsDNA target because of the tight interaction of dCas9 with the target dsDNA.
    c. dCas9 cannot be targeted to the coding region of loci to inhibit RNA Polymerase in the elongation phase of transcription.
    d. None of the above

8. What component of CRISPR Cas9 binds to cRNA to form an active complex?
    a. tracrRNA
    b. sgRNA
    c. Cas9
    d. Repair template

9. Which of the following statements about the PAM sequence is correct?
    a. Cas9 recognizes the PAM sequence on the host genome.
    b. The SpCas9 PAM sequence is 3'-NGG-5'
    c. The SpCas9 sequence occurs every 20 base pairs.
    d. None of the above

10. Which of the following statements about the variants of CRISPR-Cas9 is correct?
    a. There are versions that are photoactivatable and was created by combining light-responsive protein partners with an activator domain and a dCas9 for gene activation.
    b. An allosteric Cas9 that activate binding and cleavage when 5-hydroxytamoxifen is added.
    c. The Rapamycin-inducible split-Cas9 system was developed by combining four constructs of split Cas9 with FRB and FKBP fragments.
    d. None of the above

11. Spacer acquisition in Type I system requires the overexpression of what?
    a. Cas1
    b. Cas2
    c. Cas1 and Cas2
    d. None of the above

12. What systems are metal-dependent endonucleases?
    a. Cas1
    b. Cas2
    c. Cas1 and Cas2
    d. None of the above

13. What system binds tightly to dsDNA in a sequence-independent manner?
    a. Cas1
    b. Cas2
    c. Cas1 and Cas2
    d. None of the above

14. What two systems are most similar?
    a. Type I and II
    b. Type II and III
    c. Type I and III
    d. None of the above. They are not similar in anyway.

15. What system requires minimal Cas machinery for immunity?
    a. Type I
    b. Type II
    c. Type III
    d. All of the above

Labeling: Refer to **Figure 2.1** in the image packet. Each letter is worth **one** point. **X, Y, and Z** refer to the overall CRISPR-Cas 9 structures. **(25)** **TB#3**

Diagram Based Questions: Use **Figure 2.2** to answer the following questions about Cas9-based genetic applications. Acronyms **will** be accepted for some questions. Point values of questions will be addressed. **(20)**

1. Answer the following questions on Part *I* of the diagram. (12)
    a. What mediates the genome editing in part I? (2)
    b. Label **A**. (2)
    c. **B** refers to a pathway that can repair chromosomal breaks. What is this pathway? (2)
    d. **C** refers to the pathway with the editing template. What is this pathway? (2)
    e. **D** refers to the delta symbol (Δ). What does this symbol represent and what type of mutations can it introduce? (4)

2. Answer the following questions on Part *II* of the diagram. (4)
    a. In this editing technique, the RuvC and HNH nucleolytic active sites are mutated. In this case, Cas9 can be converted to what protein? (2)
    b. The protein in part (a) can be directed to bind to promoter sequences to interfere with the transcription initiation of E (in the diagram). What is the protein labeled **E**? (2)

3. Answer the following question on Part *III* of the diagram. (4) **TB#4**
    a. The protein described in question 2(a) can be also used as a RNA-guided DNA binding protein with additional functions. **F** addresses the functional domain, which can act in various ways. State two examples of activities that can be done by this functional domain. (4)

Short Answer Directions: Answer the short answer questions below. The point values are indicated by the number in the parentheses. Acronyms **will** be accepted for some questions. Complete sentences are **not** required for this section. **(38)**

1. Cytidine deaminase is an enzyme that can interact with CRISPR-Cas9. Answer the following enzymes about this enzyme. (4)
    a. In humans, what gene encodes this enzyme? (1)
    b. The homotetramer that is formed by cytidine deaminase catalyzes a hydrolytic deamination. What are the components involved in this reaction? (3)

2. CRISPR-Cas 9 is now being utilized in genetic engineering. The following questions refer to this system. (5)
    a. Why is CRISPR-Cas9 editing more cost-effective and efficient than other genome editors? (2)
    b. DSBs are commonly repaired by a process called NHEJ. What does NHEJ stand for? (1)
    c. True or False: NHEJ is a process that is prone to error. (1)
    d. Choose the most appropriate answer option for this question: The binding of a PAM (upstream, downstream) of the target locus assists in the direction of Cas9-mediated DSBs. (1)

3. There are different types of CRISPR. The following questions refer to these types and their aspects. (6)
    a. In what type(s) of CRISPR are the pre-crRNA transcript cleaved within the CRISPR-associated ribonucleases? (2)
    b. In what type of CRISPR are crRNA intermediates further processed at the 3' end? (2)
    c. In what type of CRISPR do the crRNAs associated with Csm or Cmr complexes? (2)
    d. What two systems both rely on the Cas6 family of endoribonucleases in the process of cleaving repeat sequences of crRNA precursor? (2)
    e. Which subtype of the Type III CRISPR-Cas system cleave DNA molecules *in vivo?* (2)

4. There are multiple studies conducted on the CRISPR system that revealed the differences between the enzymes in the *cas* gene cluster and the CRISPR systems. Answer the following questions based on these studies. (10) **TB#5**
    a. Moineau is one of the scientists that conducted studies in *Streptococcus thermophilus* and the *cas* gene cluster. These scientists discovered that there is only one enzyme in the *cas* gene cluster that can mediate target DNA cleavage. What is the enzyme? (3)
    b. In Charpentier et.al's study, it was discovered that in type II CRISPR systems, there is a noncoding component that hybridizes with crRNA to facilitate the RNA-guided targeting of Cas9. What is this component? (3)
    c. Marraffini and Sontheimer studied the differences in the structure of the CRISPR types. Answer the following questions below based on this study.
        i. For what type(s) of CRISPR would a lack of PAM within the direct repeat sequence within the CRISPR array prevent CRISPR self-targeting? (4)
        ii. For what type(s) of CRISPR is a mismatch between 5' crRNA end and the DNA target required for plasmid interference? (4)

5. There are three steps in the CRISPR-Cas immune systems. Answer the questions about these steps. (8)
    a. What is the correct order of the steps? (2)
    b. Describe the first step. (2)
    c. Describe the second step. (2)
    d. Describe the third step. (2)