

Protein Modeling Division C - Master Key

Plymouth Canton Invitational 2020

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Instructions and Clarifications:

- You have **50** minutes to finish this exam. This exam contains **two** sections: Section I - General Biochemistry and Section II - CRISPR-Cas Systems.
- There is **no** computer exploration portion for this invitational.
- Each **participant** may bring **one** 8.5" x 11" sheet of paper that contains information about this event without any annotations or labels affixed along with writing utensils for each participant.
- You **may** split the exam but you are responsible for placing them in the correct order afterwards.
- The tiebreakers for this exam are in the following order: overall score in Section I and Section II.
- If you have any comments or questions about this exam, feel free to contact us at the emails below. **Happy testing!**
- **Disclaimer:** The authors do not take credit of the images used in this exam. Credits are attributed to the original owners.

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Section I: General Biochemistry

²**Multiple Choice:** Choose the most appropriate answer option for the following questions. Each question is worth **two** points. **(40)**

- Which of the following statements about the central dogma of molecular biology is true?
 - Information is transferred from RNA to DNA to proteins.
 - Information is transferred from RNA to proteins to DNA.
 - Information is transferred from DNA to RNA to proteins.
 - None of the above
- Which of the following statements about protein structure is true?
 - It is composed of amino acids that are branched and forms rings in a linear manner.
 - The carboxylic acid group determines the identity of the amino acid.
 - A zwitterion forms at physiological pH.
 - None of the above
- How do amino acids polymerize?
 - Peptide bonds
 - Hydrogen bonds
 - Phosphodiester bonds
 - None of the above
- Which of the following amino acids have a chiral center at their alpha-carbons?
 - Tyrosine
 - Proline
 - Glycine
 - None of the above
- Which of the following statements about the side chains of proteinogenic amino acids are true?
 - There can be a side chain with just one single hydrogen atom.
 - There can be an indole heterocycle in a side chain.
 - There can be polar amino acid side chains.
 - All of the above
- How many major types of post-translational modifications to a protein are there?
 - 1
 - 2
 - 3
 - 4
 - 5

7. Which of the following statements about protein folding is true?
- They acquire their structure by spontaneously folding.
 - The proteins fold in a maximum energy configuration.
 - No proteins require no external factors in order to fold.
 - None of the above
8. What can happen in protein denaturation?
- Proteins lose their structure when put in an unsuitable environment.
 - Denatured proteins still have a defined structure but aggregate into insoluble masses.
 - This is an exotic event and usually does not happen.
 - None of the above
9. Which of the following statements about peptide bonds are true?
- They are a type of an amide group based from an organic chemistry perspective.
 - It consists of a carbonyl group's carbon atom directly bound to the nitrogen atom of a primary amine.
 - Peptide chains do not have an unbound amino group at one end.
 - None of the above
10. Which of the following has a correct match between the term and the definition?
- Oligopeptide - two amino acid molecules covalently joined through a substituted amide linkage.
 - Polypeptide - many oligopeptides
 - Peptide bond - few amino acids in a chain
 - None of the above
11. Select the correct description regarding disulfide bonds.
- They form between the sulfur atoms of two methionine side chains in a protein.
 - This reaction involves a reversible oxidation of sulfhydryl groups.
 - These typically form on the surface of proteins because disulfide bonds tend to form in reducing environments like the cytoplasm.
 - None of the above
12. Which statement below correctly describes a process in 2-Dimensional Electrophoresis (2-DE)
- This can be used to experimentally determine a protein's pI.
 - The second dimension of 2-DE is the combination of proteins by MW using a SDS-gel.
 - The proteins of a cell lysate are applied to a pKa immobilized gradient strip, upon electrophoresis the proteins migrate to their pH within the strip.
 - None of the above

13. Which of the following correctly describes the isoelectric point?
- It is the pH-value in which a protein is charged.
 - It is the pH-value in which a protein is neutral and has a zero net charge.
 - It is the pH-value where a protein has all bases deprotonated and all acids protonated.
 - None of the above
14. Considering Levinthal's paradox, if a fairly small protein has 100 amino acids and each amino acid residue has only 3 possible conformations, how many years would it take to try all of the possible conformations if it takes 10^{-13} seconds to try one conformation?
- 10^{10}
 - 10^{27}
 - 10^{34}
 - 10^{99}
15. Which statement is related to protein modules?
- It is the elements of folding that do not neatly fit into the category of tertiary structure.
 - It is the overall shape of a single protein molecule.
 - When mapped on the sequence and depicted in the relative position they have to each other, protein modules define the topology of a protein.
 - None of the above
16. The genome of the rabies virus is a negative-sense RNA. Negative sense means that the nucleic acid sequence is complementary to mRNA. What enzyme must be present in order for this virus to produce its viral proteins and replicate?
- DNA-dependent DNA polymerase
 - RNA-dependent RNA polymerase
 - DNA ligase
 - DNA-dependent RNA polymerase
 - Restriction endonuclease
17. The two functional groups found on every amino acid are.
- amino group ; carbonyl group
 - amino group ; carboxyl group
 - carboxyl group ; phosphate group
 - carboxyl group ; hydroxyl group
 - phosphate group ; amino group

18. How is water used in hydrolysis by organisms?

- a. To break down protein molecules
- b. To synthesize new protein molecules
- c. To re-hydrate proteins in dormant organism (like seeds)
- d. To aid transcription of DNA to RNA

5' ATCAGCGCTGGC 3'

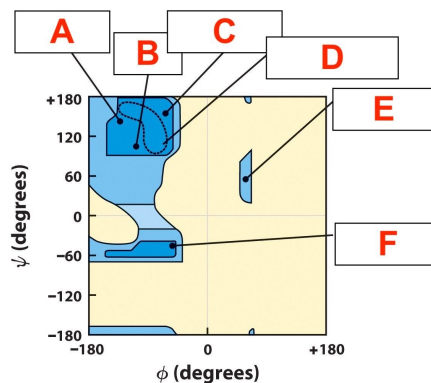
19. The above sequence of DNA is part of a gene. How many amino acids are coded for by this segment?

- a. 4
- b. 8
- c. 12
- d. 20
- e. 1

20. Which transition metal and oxidation state is used by APOBEC3A deoxycytidine deaminases protein?

- a. Zinc 1⁺
- b. Zinc 2⁺
- c. Zirconium 2⁺
- d. Zirconium 3⁺
- e. Zirconium 4⁺

Labeling: Label the diagram below. Each letter is worth **three** points. (18)



- A. Antiparallel beta sheets
- B. Parallel beta sheets
- C. Collagen triple helix
- D. Right-twisted helix
- E. E. Left-handed alpha helix
- F. F. Right-handed alpha helix

²**Diagram-based Questions:** Use the provided diagrams to help you answer some of the following questions. The point values will be denoted for each question. (58)

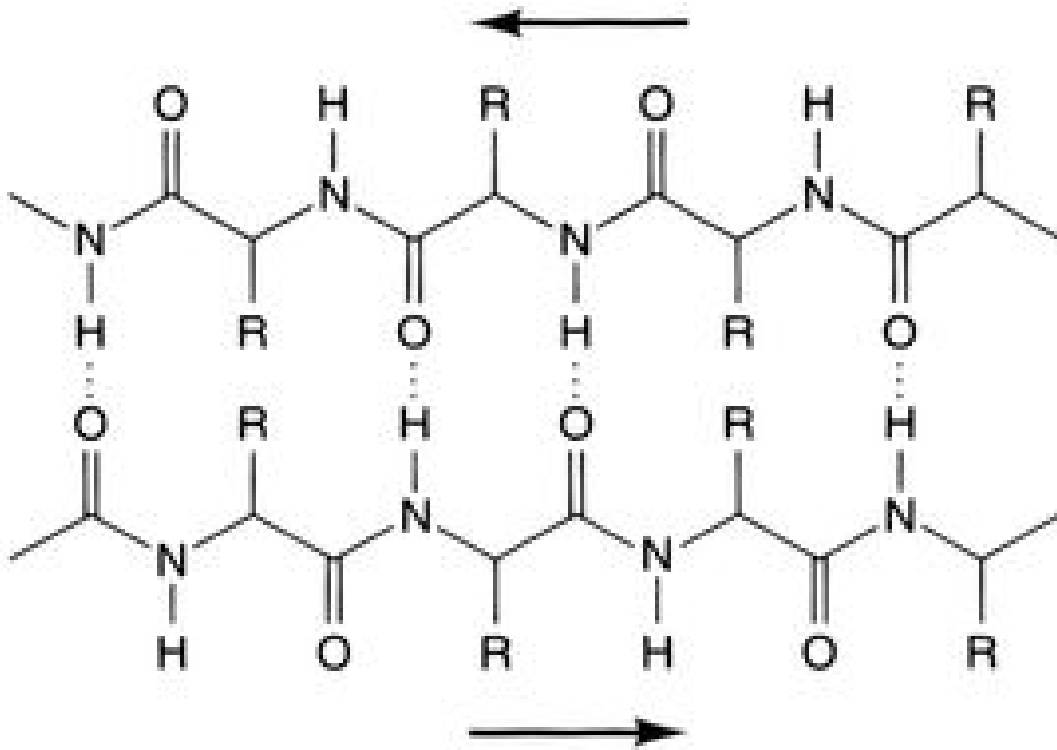


Figure 1 (24 points)

1. What is the name of the structure in the image? (2) **beta-sheet, beta-pleated sheet**
2. Who first proposed this structure in 1951? (4) must provide both names for full credit - **Linus Pauling and Robert Corey**
3. Why is the probability of bulky steric clashes lessened in this structure? (4) **the amino acid chain is almost fully extended throughout the beta-strand**
4. Provide the range for the optimal conformation regarding the angle phi in this structure (as shown in a Ramachandran plot). (4) **-120 to -60 degrees (2 points per correct number)**
5. Provide the range for the optimal conformation regarding the angle psi in this structure (as shown in a Ramachandran plot). (4) **120 to 160 degrees (2 points per correct number)**
6. Some sequences involved in this structure, when traced along the backbone, can take what type of turn in orientation/direction, sometimes through one or more prolines? (4) **hairpin turn**
7. **True or False:** There are only two types of forms for this structure: parallel and antiparallel. (2) **False (explanation: there can be mixed forms.)**

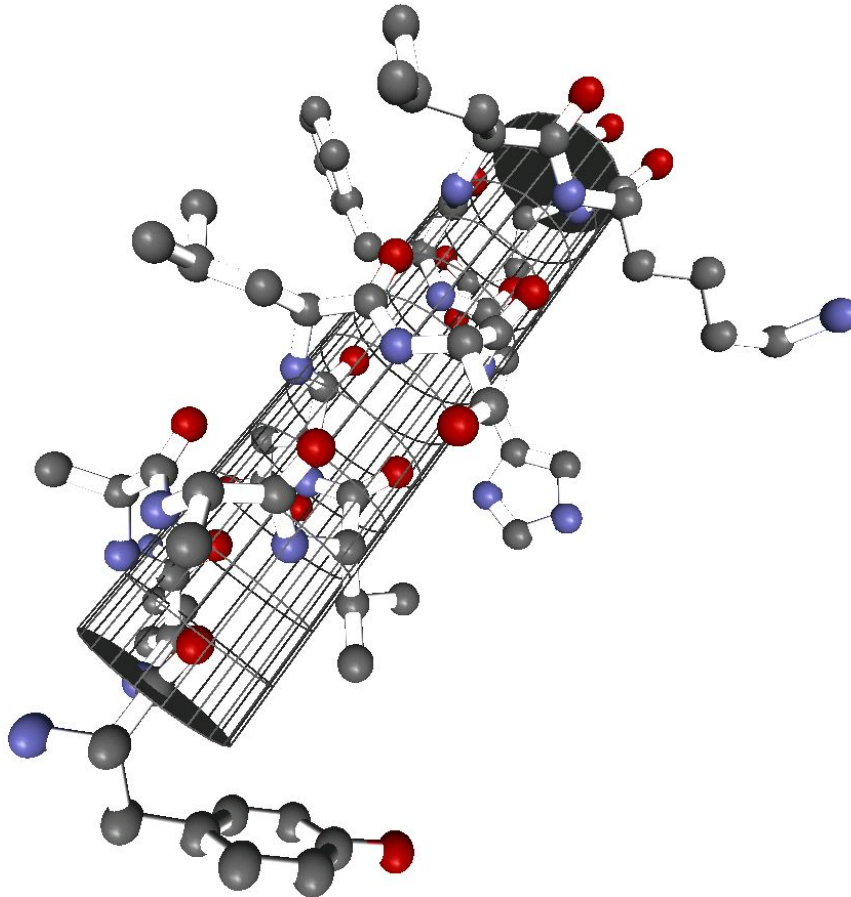


Figure 2 (34 points)

8. What is the name of the structure shown in the image? (2) **alpha helix**
9. Why is this structure always right-handed in natural proteins? (6) **the main chain atoms from residues spaced four residues apart hydrogen bond with one another**
10. How many amino acids are there per turn of this structure? (4) **3.6**
11. There is distribution of a specific kind of residue that tend to occur primarily on one face of this structure. Describe this kind of residue in one word. (4) **hydrophobic**
12. Why are alpha helices often capped at the N-terminal end by a negatively charged amino acid like glutamic acid? (10) **10 points for full explanation, 5 points for partial explanation, 0 points for incorrect explanation - a helix has a buildup of positive charge at the N-terminal end and negative charge at the C-terminal end, which is a destabilizing influence for the helix; the capping of the N-terminal end with a negatively charged amino acid stabilizes the helix dipole**
13. State an amino acid that would be involved in C-terminal capping. (4) **give credit for any positively charged amino acid like lysine**
14. **True or False:** There is almost no free space within this structure. (2) **True**
15. **True or False:** Proline is an example of an amino acid that can disrupt this structure. (2) **True**

²**Identification:** Using the provided images, **identify** the structural motif shown. Then, answer the following questions about this structural motif in the following page. Point values will be assigned for each question. (**Clarification:** All of the images correspond to the **SAME** structural motif.) (60)

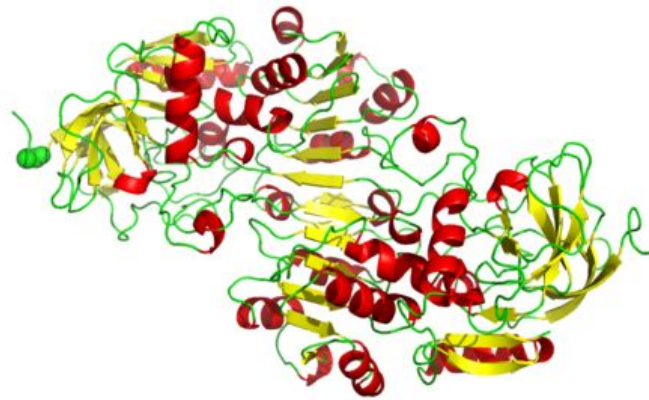


Figure 3: Illustrates beta-alpha folding in alcohol dehydrogenase

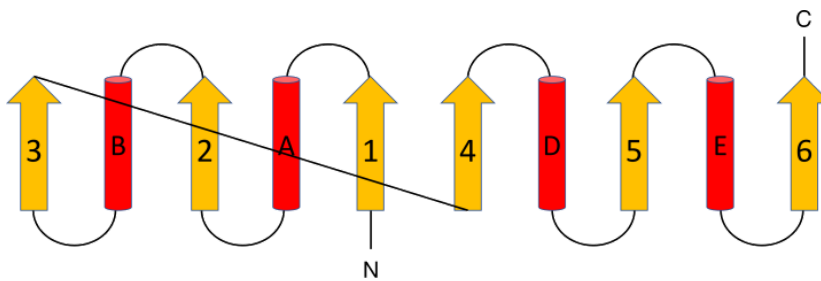


Figure 4: Schematic diagram of a six-stranded version of this motif

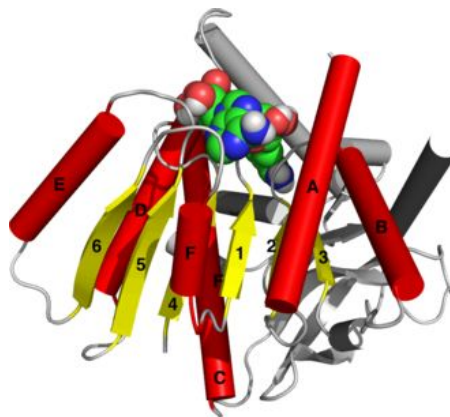


Figure 5: Front view of the structural motif from *E. coli* malate dehydrogenase (5KKA)

²Identification (continued)

16. What structural motif is shown in Figures 3-5? (5) **Rossmann fold**
17. This structural motif is found in proteins that bind what structures? (**Hints:** enzyme cofactors FAD, NAD⁺, and NADP⁺) (5) **nucleotides**
18. Based on the provided figures, what is the pattern between the beta strands and alpha helical segments? (5) **alternating**
19. In this structural motif, the beta strands are hydrogen bonded to each other. What structure forms as a result of the beta strands bonded to each other? (5) **(extended) beta sheet**
20. How many parallel beta strands are there in this structure? (5) **6**
21. The first three strands are connected by what structure? (5) **alpha helices**
22. What is the topology of the strands in this structural motif? (**Hints:** The answer to this question involves numbers, and questions 23 + 24 could help with answering the questions.) (10) **32145**
23. In question 22, the number 1 would represent what part of the strands? (5) **N-terminal**
24. In question 22, the number 6 would represent what part of the strands? (5) **C-terminal**
25. Describe the overall tertiary structure of this structural motif. (10) (**Hint:** Think about a sandwich). **10 points for a complete explanation, 5 points for partial explanation, 0 points for incorrect explanation - three-layered "sandwich" wherein the "filling" is composed of an extended beta-sheet and the two "slices of bread" are formed by the connecting parallel alpha helices**

Section II: CRISPR-Cas Systems

²**Multiple Choice:** Choose the most appropriate answer option for the following questions. Each question is worth **two** points. **(28)**

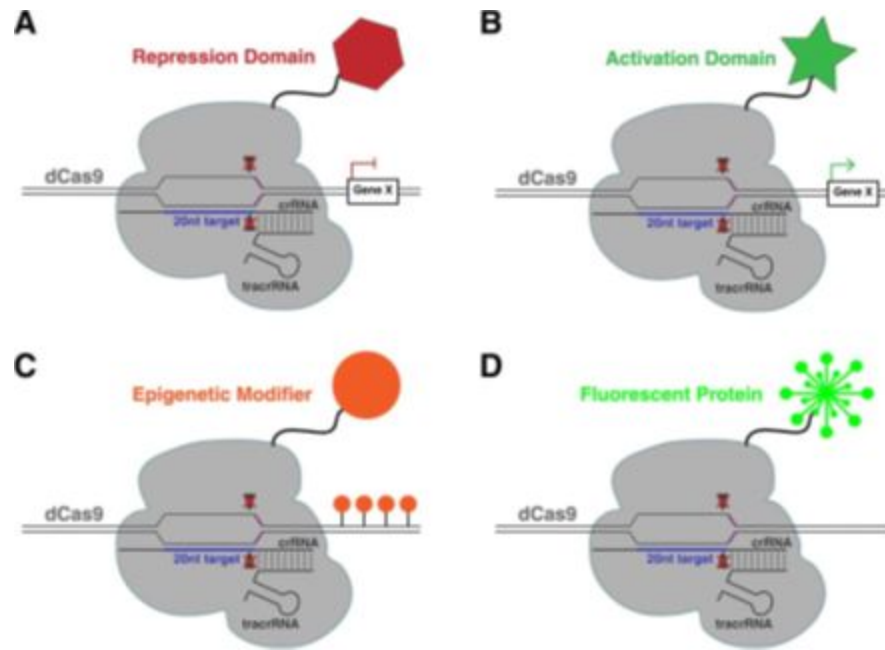
1. Which CRISPR system type(s) consists of the Cas9 nuclease?
 - a. I
 - b. II**
 - c. III
 - d. More than one of the above
 - e. None of the above
2. Which CRISPR system type(s) requires the annealing of the tracrRNA to the repeat sequences of the pre-crRNA and the subsequent cleavage of dsRNA by host RNase III?
 - a. I
 - b. II**
 - c. III
 - d. More than one of the above
 - e. None of the above
3. Which CRISPR system type(s) involves primary processing of the pre-crRNA using Cas6 endoribonuclease within the Cascade complex?
 - a. I**
 - b. II
 - c. III
 - d. More than one of the above
 - e. None of the above
4. In Type III CRISPR-Cas systems, Cas6 cleaves the pre-crRNA to generate int-crRNAs that can be incorporated into what complex?
 - a. Cmr/Cas10
 - b. Csm/Cas10
 - c. mat-crRNAs
 - d. A and B**
 - e. None of the above
5. Which two symptoms are considered to be most similar to each other?
 - a. I and II
 - b. I and III**
 - c. II and III
 - d. None of the above - they are all completely different.

6. Which of the following statements about Cas9 nucleases is correct?
- It carries out strand-specific cleavage by only using the conserved HNH nuclease domain.
 - An aspartate-to-alanine (D10A) mutation in the RuvC catalytic domain permits the Cas9 nickase mutant in the RuvC catalytic domain to yield single-stranded breaks.
 - The specificity of target recognition can be increased by offsetting sgRNA pairs, which in turn guides Cas9n to nick both strands of a target locus to mediate a DSB.
 - More than one of the above
 - None of the above
7. Which of the following is a limitation of the Cas9 system and its implications in genetic engineering?
- The only requirement for Cas9 target site selection is the presence of a PAM sequence directly 5' of the 20-bp target sequence.
 - The only requirement for Cas9 target site selection is the presence of a PAM sequence directly 3' of the 20-bp target sequence.
 - The only requirement for Cas9 target site selection is the absence of a PAM sequence directly 5' of the 20-bp target sequence.
 - The only requirement for Cas9 target site selection is the absence of a PAM sequence directly 3' of the 20-bp target sequence.
 - None of the above
8. How many subtypes of CRISPR-Cas systems are there?
- 3
 - 6
 - 9
 - 12
 - 20
9. Choose the answer choice that contains a correct match between the term and the definition.
- Adaptation - CRISPR arrays are transcribed and processed into small interfering crRNAs
 - crRNA biogenesis - new spacers are acquired from exogenous nucleic acid into the CRISPR locus
 - Targeting - crRNAs guide Cas nucleases for specific cleavage of homologous sequences
 - More than one of the above
 - None of the above

10. What is the term for the sequence in the exogenous nucleic acid that corresponds to a CRISPR spacer?
- PAM
 - Protospacer
 - Cas9
 - crRNA
 - None of the above
11. Which of the following is a possible application of CRISPR-Cas immunity?
- Phages acquiring CRISPR-Cas systems to target host defense systems
 - Barrier against horizontal gene transfer
 - Cas9 repressing an endogenous lipoprotein gene to promote pathogenesis by preventing host proinflammatory response against the protein
 - More than two of the above
 - None of the above
12. CRISPR-Cas interference complexes, exemplified by Cascade and Cas9, form what structures?
- PAM
 - cr-RNAs
 - New *cas* genes
 - Nucleoprotein R-loops
 - None of the above
13. What is considered to be the most conserved protein that is present in most of the CRISPR-Cas systems?
- Cas1
 - Cas2
 - Cas9
 - Cas10
 - None of the above - they are all present in the same amount.
14. Which signature gene in a specific CRISPR-Cas system encodes a multidomain protein that combines all the functions of effector complexes and the target DNA cleavage and is also essential for the maturation of the crRNA?
- Cas1
 - Cas2
 - Cas9
 - Cas10
 - None of the above

¹The following short answer questions are specific to the CRISPR-Cas Systems. Each question is 2 points each unless stated differently. (17)

In the figure below is shown potential methods for Cas9 targeting to a specific sequence in the genome. For the following two questions choose a or b or c or d corresponding to the letters of the method in the figure below or e (none of the methods).



15. Which method can be used to block transcription from a gene? ___ **A**

16. Which method can be used to change the histone code? ___ **C**

17. Which method can be used for cytidine deaminase? ___ **E**

Answer E is none of the above depictions.

18. CRISPR is an acronym. What does it stand for?

Clustered Regularly Interspaced Short Palindromic Repeat

19. What is a palindrome?

Nucleic acid whose sequence read in a 5' to 3' direction is exactly reversed in the 3' to 5' direction.

20. Why are palindromes (a) important and (b) what is an example of their use? (3 points)

They are recognized (specifically bound) by DNA interacting proteins such as restriction enzymes and other proteins.

Or they form hairpin loops when in the single strand form as in CRISPR RNA.

Other examples too (Snurps, rRNA, tRNA, etc.)

21. In bacteria the normal functions of Cas9 is to:

- a. recognize and digest viral DNA.
- b. prevent viral mRNA from being used.
- c. Make gene edits in plasmid DNA.
- d. Replicate bacterial genome.

22. Within bacteria how is the CRISPR/Cas9 system action like the action of restriction enzymes?

- a. Both recognize 16 base pair sequences.
- b. Both use guide RNA sequences.
- c. Both originated as mutations in viruses.
- d. Both make double strand cuts in DNA.
- e. Both require that the target DNA form hairpin double strand sequences.