Exploring the World of Science

University of Michigan Science Olympiad
2021 Invitational Tournament

Protein Modeling C

Test length: 50 Minutes

Team name: _____ **Team number:** _____

Student names: _____

Welcome to the **University of Michigan 2021 Invitational Protein Modeling** test! Make sure you have a stable internet connection and are ready to compete!

For this test, you are allowed the following resources:

- A Google Meet/Zoom/Skype/Phone/Video call with your partner
- A cheat sheet/binder, printed or in pdf format on your computer
- Programmable/Non-programmable calculator
- Scratch paper

You **MAY NOT** take advantage of the following resources. Doing so will result in a disqualification plus 30 points added to your team's overall score.

- ANY internet resource (other than linked resources within the test)
- Help from any person other than your partner
- A printed version of the test

This event requires a pre-build. You may view the pre-build instructions here https://docs.google.com/document/d/1NQ8rOeijZXYB54KCKXodD6gwaHsJwxqf2Rb8jLAisk8/edit?usp=sharing
and submit here:https://forms.gle/wFaiaKLLim6FoPnq9 . If you have not already done so, please turn in your pre-build using this google form by the END OF YOUR TIME SLOT.
This test consists of 75 questions and you will have 50 minutes to complete it.

The Tiebreakers for this test will be: **Part 2 Q20, Part 3 Q50, Q55, Q61, Q66**

If you experience technical difficulties during the test:

- Immediately contact the event supervisor through the classroom feature on Scilympiad, stating clearly what issue you are having.
- If your work is not saving/submitting, take screenshots of your answers on Scilympiad and submit them to this google form. Try to stay within your allotted 50 minutes.

This event requires a pre-build. You may view the pre-build instructions here [EMBED LINK

TO PDF/GOOGLE DOC]. If you have not already done so, please turn in your pre-build using this google form by the END OF YOUR TIME SLOT.

PART 1: PREBUILD - 20 points

The protein that the competitors will model is residues 31-145 of Chain A of the APOBEC3A Cytidine Deaminase Protein as indicated by the rules and the MSOE (national sponsor for protein modeling).

Students will submit a document containing images of their prebuild model along with a notecard detailing their creative additions through a google form: https://forms.gle/wFaiaKLLim6FoPnq9

Further instructions about what images should be submitted can be found on this google document: https://docs.google.com/document/d/1NQ8rOeijZXYB54KCKXodD6gwaHsJwxqf2Rb8jLAisk8/edit?usp=sharing

PART 2: JMOL EXPLORATION - 35 points

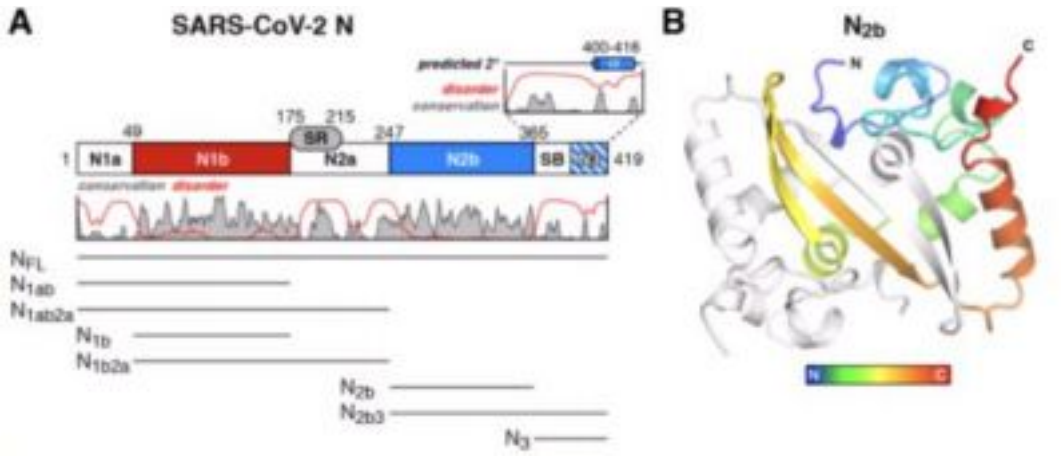
"In December 2019, a new type of coronavirus (SARS-CoV-2 or 2019-nCoV) causing a novel pneumonia now named COVID-19 broke out in Wuhan, China. The SARS-CoV-2 genome is composed of approximately 30,000 nucleotides, which encodes four structural proteins including spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein. The N protein is a highly immunogenic and abundantly expressed protein during infection. Furthermore, the N protein is frequently used in vaccine development and serological assays. At present, there are few reports focusing on SARS-CoV-2 N protein, and the updated understanding of SARS-CoV-2 N protein is in urgent need." *

Zeng, Weihong et al. “Biochemical characterization of SARS-CoV-2 nucleocapsid protein.” Biochemical and biophysical research communications vol. 527,3 (2020): 618-623. doi:10.1016/j.bbrc.2020.04.136,https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7190499/

"As the most prominent surface component of the virus, the spike protein is the major target of antibodies in patients, and is the focus of several current efforts at SARS-CoV-2 vaccine development. Initial trials using antibody-containing plasma of convalescent COVID-19 patients has also shown promise in lessening the severity of the disease.While the above efforts target viral entry, RNA synthesis, and protein processing, there has so far been less emphasis on other steps in the viral life cycle. One critical step in coronavirus replication is the assembly of the viral genomic RNA and nucleocapsid (N) protein into a ribonucleoprotein (RNP) complex, which in betacoronaviruses like SARS-CoV-2 is thought to form a helical filament structure that is packaged into virions through interactions with the membrane-spanning membrane (M) protein. Despite its location within the viral particle rather than on its surface, patients infected with SARS-CoV-2 show higher and earlier antibody responses to the nucleocapsid protein than the surface spike protein. As such, a better understanding of the SARS-CoV-2 N protein’s structure, and

structural differences between it and N proteins of related coronaviruses including SARS-CoV, may aid the development of sensitive and specific immunological tests."

*Ye, Qiaozhen et al. "Architecture and self-assembly of the SARS-CoV-2 nucleocapsid protein." *bioRxiv : the preprint server for biology* 2020.05.17.100685. 17 May. 2020, doi:10.1101/2020.05.17.100685. Preprint. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7263487/



Now that you have some background information of the nucleocapsid protein of the SARS-CoV-2 genome, we will delve deeper into the structure of SARS-CoV-2 Nucleocapsid dimerization domain.

Protein Bank: https://www.rcsb.org/structure/6WZO
Research/Experiment: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7263487/ Online version of JMOL: https://cbm.msoe.edu/markMyweb/jmolDesignEnvironment/#forward

To answer the following questions, you have access to the above protein bank link, the pdb file on jmol (available for download in the protein bank link), and the research/experiment paper. **To access the structure on jmol**, press the tab labeled "Load a new structure" >> type in 6WZO in the second blank labeled "Public .PDB file (by ID)" >> press load

However keep in mind that not all questions refer to the above resources.

1. What resolution (Å) did the researchers use in this experiment to identify the structure of SARS-CoV-2 Nucleocapsid dimerization domain? (1 pt)

2. List at least one advantage and one disadvantage of using X-ray Crystallography in an experiment (be specific)? (2 pts)

Use the resolution values (Å) provided below to answer the following four multiple choice questions. Answer with the minimum resolution value necessary to observe the following situations:

I) 1.0

II) 1.5

III) 2.5

IV) 3.5

V) 4.0
VI) 4.5

3. Hydrogen atoms are visible (0.5 pts)
    a. I
    b. II
    c. III
    d. IV
    e. V
    f. VI
4. Secondary structures well defined and visible (0.5 pts)
    a. I
    b. II
    c. III
    d. IV
    e. V
    f. VI
5. Global Fold (limited view of secondary structures) (0.5 pts)
    a. I
    b. II
    c. III
    d. IV
    e. V

f. VI

6. All phi-psi angles are well defined and visible (0.5 pts)
    a. I
    b. II
    c. III
    d. IV
    e. V
    f. VI

Now for the following questions refer to either the JMOL version of the protein or the RCSB Protein Bank:

7. How many chains are present in the nucleoprotein? (1 pt)

The following questions refer to chain A of the nucleoprotein:

8. How many beta strands are present? (1 pt)
9. Classify beta strand(s) as either parallel or antiparallel? Briefly state which classification is more stable and explain why at an atomic level? (2 pts)

10. How many alpha helices are present? (1 pt)

11. There are other secondary structures present in chain A (True or False) -- answer with a capital T for true or a capital F for false. (1 pt)

12. Based on the answer to the previous question, name the secondary structure. If you answered false, enter "N/A". (1 pt)

13. Indicate the x, y, z coordinates of the C-terminus (first blank -- x coordinate, second blank -- y coordinate, third blank -- z coordinate) (1.5 pts)

14. Indicate the x, y, z coordinates of the N-terminus (first blank -- x coordinate, second blank -- y coordinate, third blank -- z coordinate) (1.5 pts)

15. Which amino acid serves as a rotamer outlier in chain A? (indicate with full amino acid name) (1 pt)

16. How many hydrogen bonds are present? (1 pt)

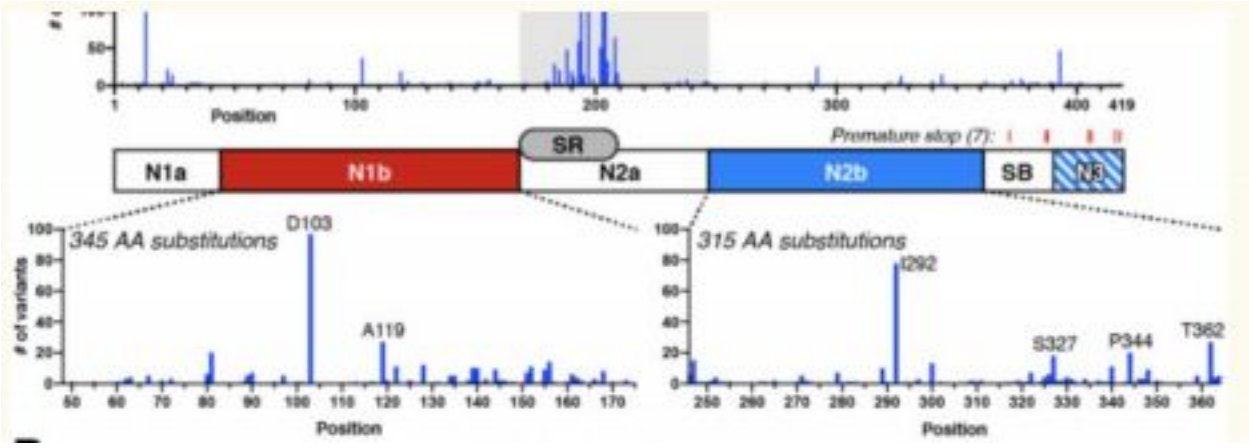
17. How many sulfur atoms are interacting with chain A? (1 pt)

The following questions require the use of the research article linked at the beginning of the section:

18. Typically nucleocapsid proteins have a general common structure with the presence of an ordered RNA binding domain (N1b) and a dimerization domain (N2b).
    a. The dimerization domain (N2b) of SARS-COV-2 is most similar to the dimerization domain of which structure? (1 pt)
    b. List the residual differences between both structures. (3 pts)

    c. Why do you think these differences/modifications are significant? (3 pts)
19. Which residues are most commonly mutated in the N2b dimerization domain? (2 pts)

20. a. What are the functionalities of the N2b domain, C-terminal spacer B/N3, N2a linker domain. What is the main drawback of targeting the N2a domain with individual patient antibodies? What conclusions can be made? (8 pts) *Tiebreaker 1*



PART 3 - GENERAL EXAM - 45 points

We have divided the general exam into three different sections, each covering a topic indicated by the National Science Olympiad Rules:

Section 1: Protein Folding Chemistry

Section 2: CRISPR Mechanisms

Section 3: APOBEC3A Cytidine Deaminase Protein

There are different formats for questions, please follow the directions. Tiebreakers will also be indicated

Section 1: Protein Folding Chemistry

1. A right handed helix is typically how long in angstroms (Å)? And each residue corresponds to a rise of _____ (Å). (0.5 pt)

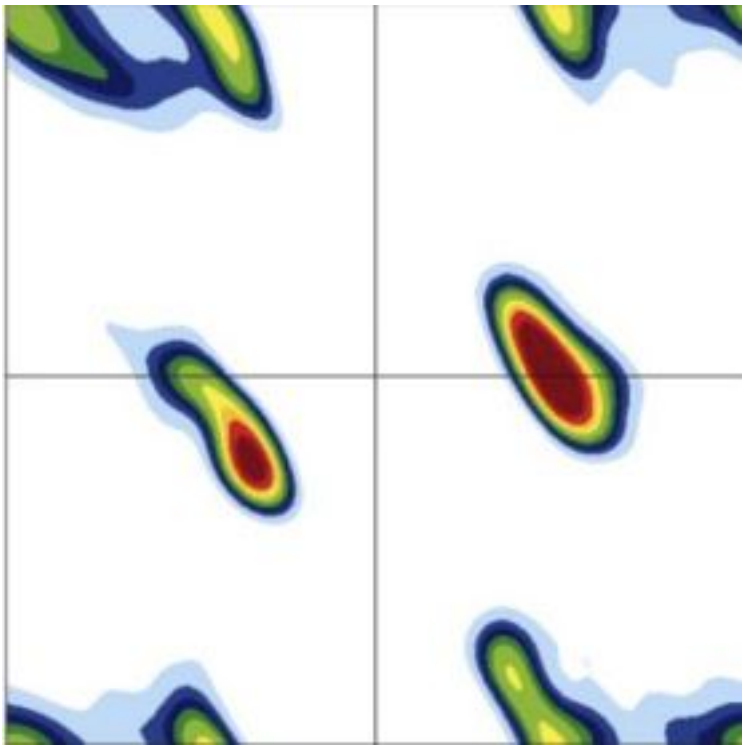
2. What is the length of a polypeptide (angstroms) with 150 amino acids in a helical shape (make up an alpha helix)? (0.5 pt)

3. Define chirality (1 pt):

4. Which of the following is a chiral amino acid (circle all that apply) (0.5 pt)
    a. Proline
    b. Glycine
    c. Histidine
    d. Tryptophan
    e. Alanine
    f. All of the above

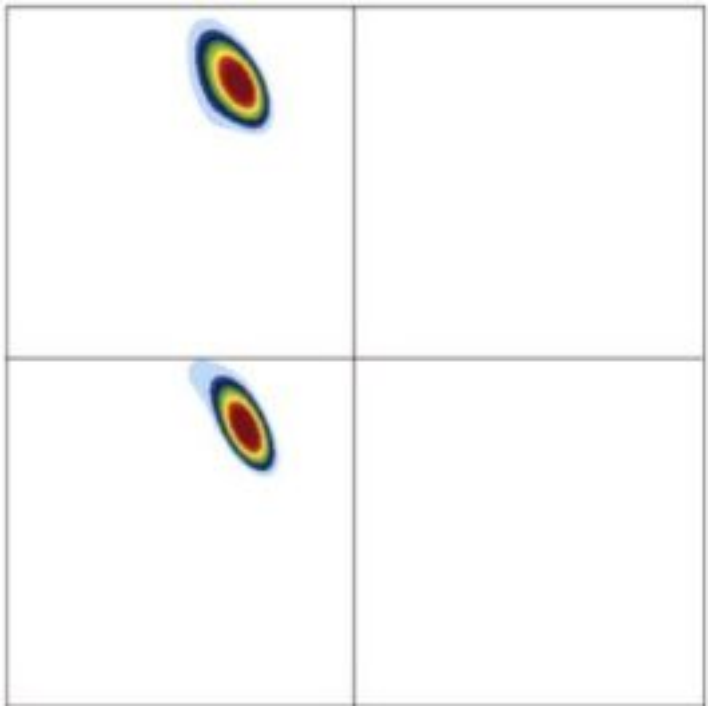
5. Which of the following is an achiral amino acid (circle all that apply)? (0.5 pt)
    a. Proline
    b. Glycine
    c. Histidine
    d. Tryptophan
    e. Alanine
    f. All of the above
6. Based on the answers to question 4 and question 5, provide structural evidence to explain why the chosen amino acids are chiral and achiral respectively. (1 pt)
7. What effect does chirality have on protein folding? (1 pt)

8. Name this plot (first blank), define the horizontal axis (second blank), define the vertical axis (third blank) -- spell out/do not use symbols (0.5 pts)



9. From the above plot image, which amino acid does it most closely resemble? (0.5 pts)

10. Briefly describe this amino acid (explain atomic structure in words), detail any important properties relevant to protein folding? (1 pt)

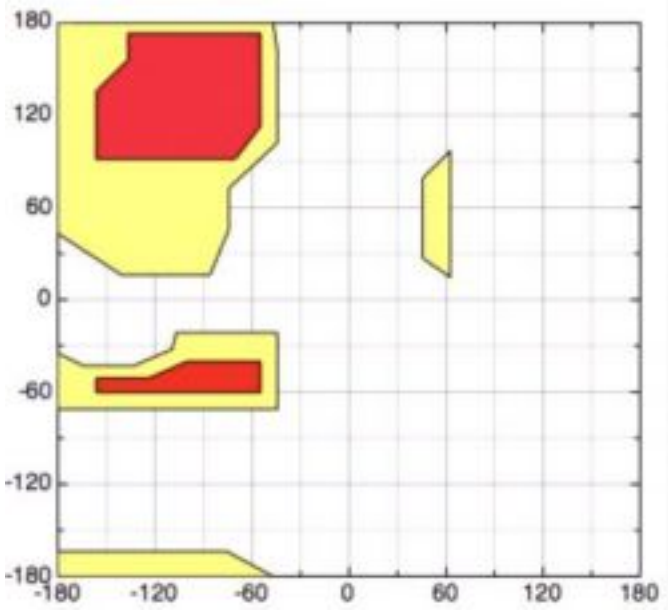


11. What amino acid does this plot resemble? (0.5 pts)

12. Briefly describe this amino acid (explain atomic structure in words), detail any important properties relevant to protein folding? (1 pt)

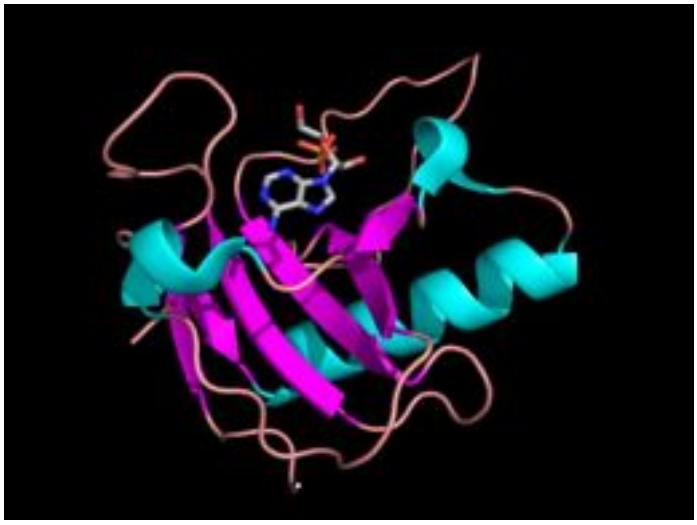
13. What is the significance of these two amino acids in each of the secondary structures (alpha helices, beta sheets, beta turns)? (1 pt)

14. Given this image, explain the differences between the red, yellow, and white colored areas. (1 pt)



15. If a plot had some density in the lower left quadrant what secondary structure would you expect to be marked? (0.5 pts)

16. Ribonuclease is a small protein responsible for splitting and controlling RNA strands. It also contains cysteine molecules connected via disulfide bonds.



What two components when combined can denature this protein (ignore general salts) (1 pts)?

17. When these two components are added, ribonuclease denatures -- describe this denatured state. (2 pt)
18. If the two components are taken out at the **same time**, ribonuclease returns to its original structure and disulfide cross-links are correctly reformed. (0.5 pts)
    a. True

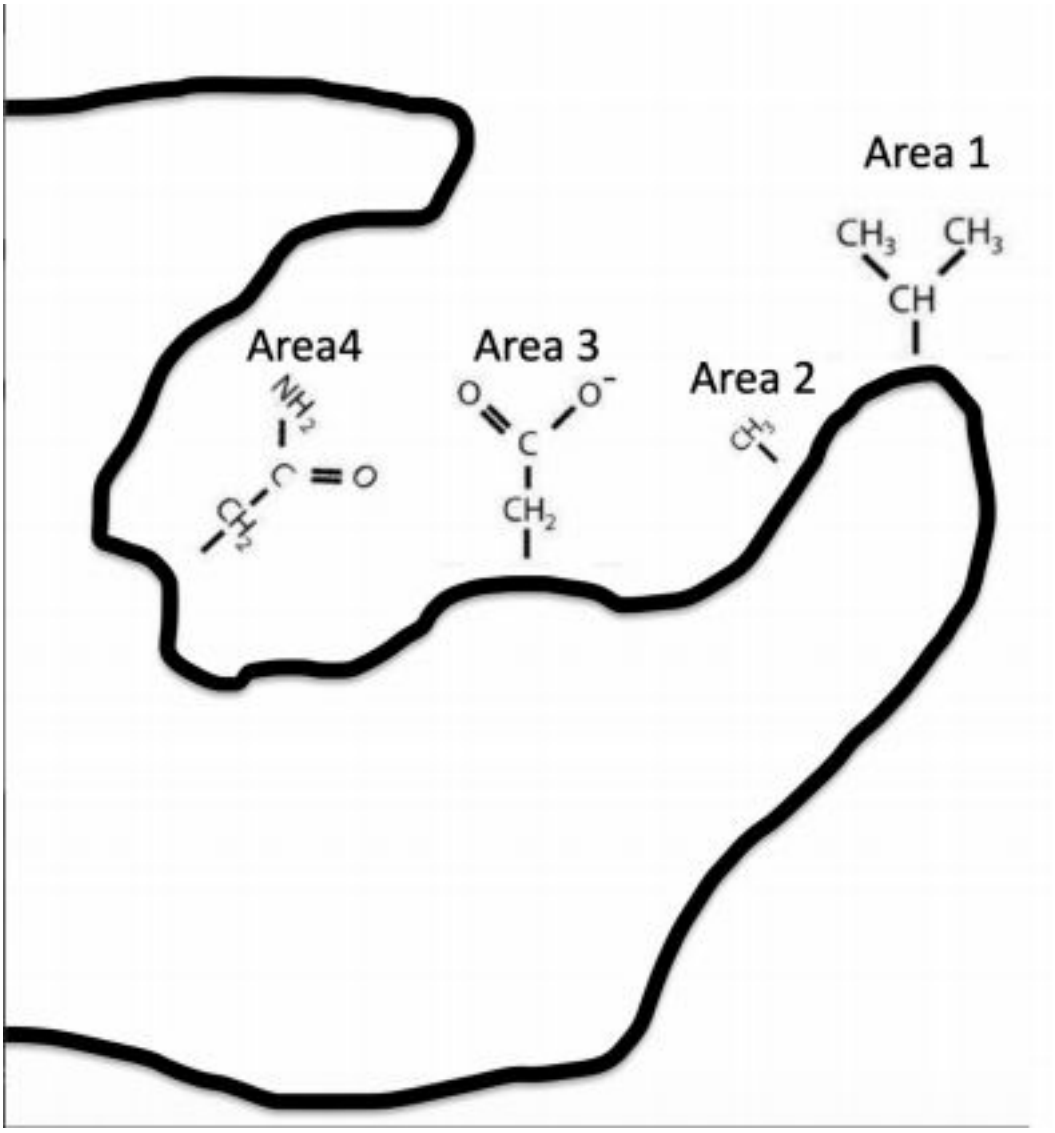
b. False

19. Which of the following protein precipitation techniques are valid (choose all that apply) (0.5 pts):
    a. Salting out
    b. Hydrophilic polymers
    c. Using acetone as a solvent
    d. Isoelectric precipitation
    e. All of the above

20. Which amino acid can form disulfide bonds? (0.5 pts)
    a. Cysteine
    b. Tyrosine
    c. Glycine
    d. Glutamine
    e. All of the above

21. Name two components that can be used in a cleaving reaction of disulfide bridges? (1 pts)

A pharmaceutical drug company has recently uncovered a protein-substrate complex. The picture below details the rough active site for the protein. Use the picture below to answer the following set of questions (23 - 31):



22. What amino acid is present in Area 1? (0.5 pts)
    a. Leucine
    b. Isoleucine
    c. Valine
    d. Serine
    e. Alanine
23. What amino acid is present in Area 2? (0.5 pts)
    a. Glycine
    b. Proline
    c. Serine
    d. Lysine
    e. Alanine
24. What amino acid is present in Area 3? (0.5 pts)

a. Glutamic Acid
b. Aspartic Acid
c. Arginine
d. Histidine
e. Asparagine

25. What amino acid is present in Area 4? (0.5 pts)
    a. Glutamic Acid
    b. Lysine
    c. Aspartic Acid
    d. Asparagine
    e. Glutamine

For the purposes of this question, the specific substrate amino acids involved with this active site and complex is unknown.

26. What is the strongest interaction/force that can exist between the Area 2 amino acid (on the drug surface) and the substrate surface? (1 pt)
    a. Hydrogen Bonds
    b. Van der Waal Forces
    c. Ionic Bonds
    d. Covalent Bonds
27. What is the strongest interaction/force that can exist between the Area 3 amino acid (on the drug surface) and the substrate surface? (1 pt)
    a. Hydrogen Bonds
    b. Van der Waal Forces
    c. Ionic Bonds
    d. Covalent Bonds
28. What is the strongest interaction/force that can exist between the Area 4 amino acid (on the drug surface) and the substrate surface? (1 pt)
    a. Hydrogen Bonds
    b. Van der Waal Forces
    c. Ionic Bonds
    d. Covalent Bonds

Amazed by your knowledge of proteins and their mechanisms, the pharmaceutical company hires you to help design a potential protein that would bind more effectively and fit better within the active site of the image above. On your first day of work, you're faced with three questions:

29. Would Serine or Isoleucine on the substrate surface allow for a greater interaction with Area 4? (0.5 pts)

a. Serine

b. Isoleucine

30. Would Glutamic Acid or Lysine on the substrate surface allow for a greater interaction with Area 3? (0.5 pts)

    a. Glutamic Acid

    b. Lysine

31. Explain your answer choices in further detail from both part a and part b. (2 pts) *Tiebreaker 2*

32. During an enzyme reaction, if the enzyme concentration is decreased by a factor of 5, how will the Km value change correspondingly? (0.5 pts)

    a. Km decreases by a factor of 5

    b. Km decreases by a factor of 2.5

    c. Km increases by a factor of 5

    d. Km increases by a factor of 2.5

    e. Km is unchanged

33. An enzyme has a Vmax of 50 mM product formed per min. The Km for the substrate of the enzyme is 10 mM. What is the initial reaction rate (v0) when [S] is 5.0 mM? (1 pts)

    a. 41.6 mM per second

    b. 16.6 mM per second

    c. 47.3 mM per second

    d. 27.6 mM per second

    e. 33.3 mM per second

    f. 14.9 mM per second

34. Which of the following combinations of choices will describe an enzyme with high catalytic efficiency? (0.5 pts)

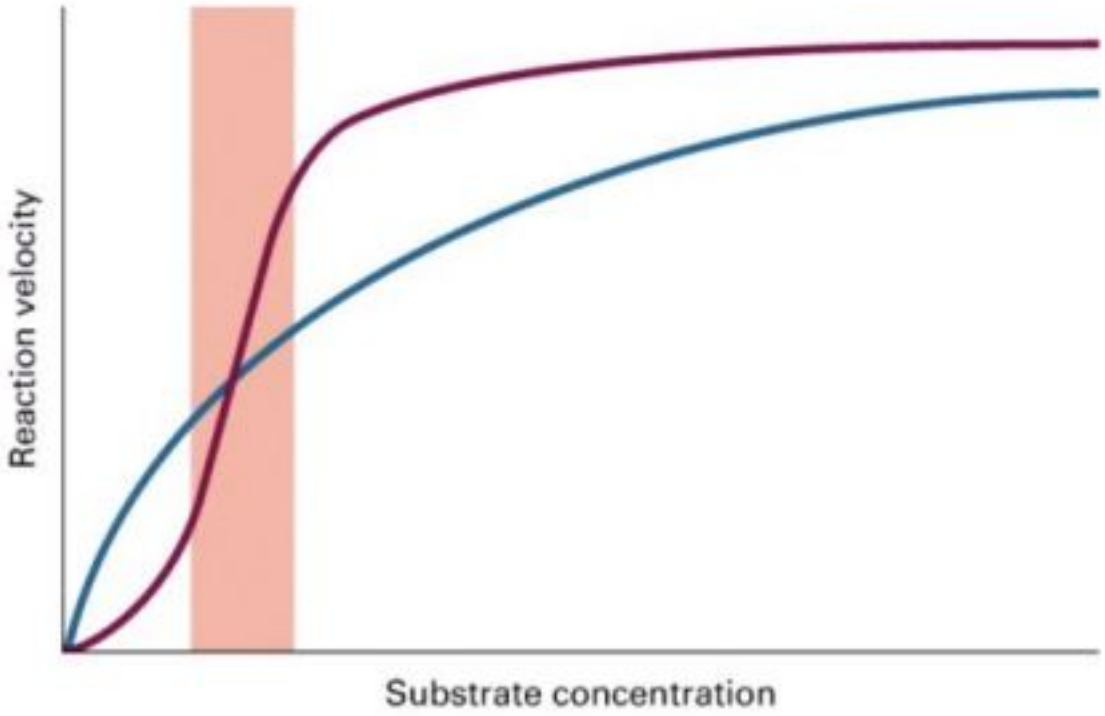
    a. Low kcat, High Km

    b. High kcat, High Km

    c. High kcat, Low Km

    d. Low kcat, Low Km

35. Select the most accurate statement regarding the purple curve (0.5 pts):



a. Enzyme shown is allosteric because graph of substrate concentration and velocity is sigmoidal

b. Enzyme shown is not allosteric because graph of substrate concentration and velocity is sigmoidal

c. Enzyme shown is allosteric because graph of substrate concentration and velocity follows the standard Michaelis-Menten curve

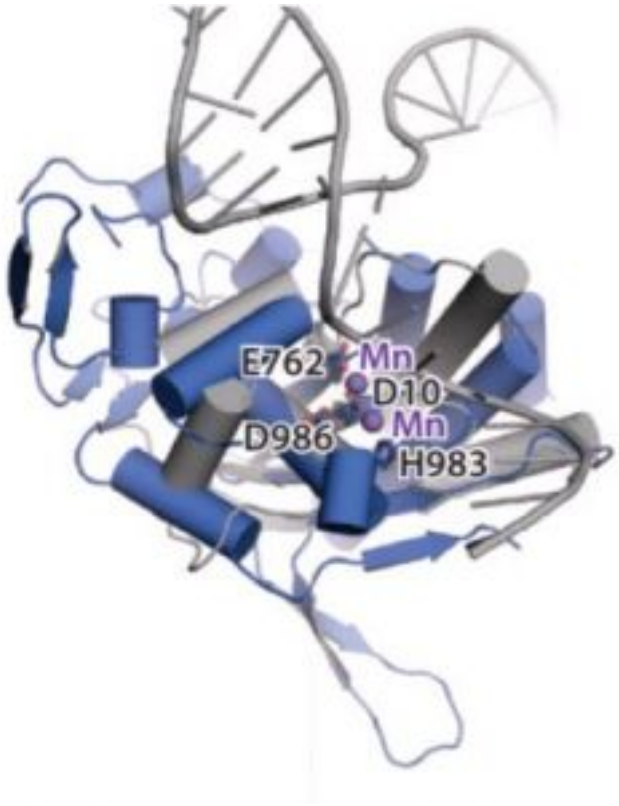
d. Enzyme shown is not allosteric because graph of substrate concentration and velocity follows the standard Michaelis-Menten curve

36. Describe the formation and structure of an Enzyme-substrate complex (be sure to address specific interactions and proximity) (2 pts) *Tiebreaker 3*

Section 2: CRISPR Mechanisms

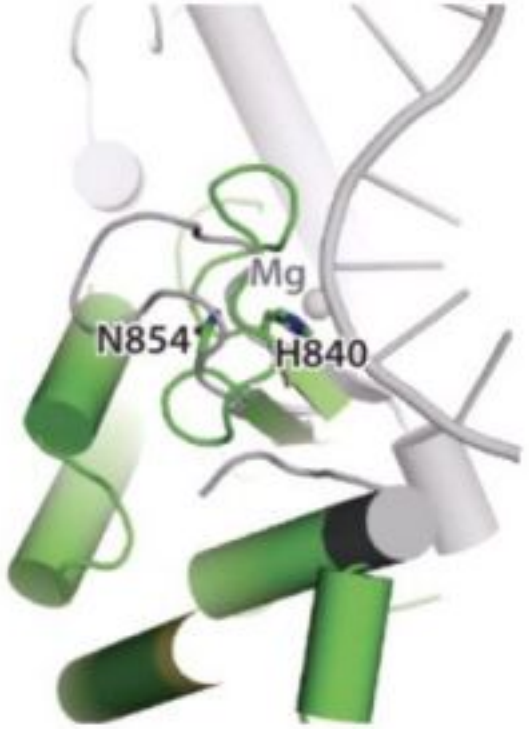
37. What are the three main steps for the CRISPR - Cas9 immune response (name of step followed by description of step)? (2 pt)

38. Name the domain involved in the CRISPR/CAS9 system diagram below: (0.5



pts)

39. Name the domain involved in the CRISPR/CAS9 system diagram below: (0.5 pts)



40. What are the functions of the two domains above and how do they differ? (1 pt)

41. Which one of the above domains is most likely to use a two-metal-ion catalytic mechanism for cleavage? (0.5 pts)

42. What is the main marker to differentiate one-metal-ion-dependent and two-metal-ion-dependent nucleic acid cleaving enzymes in terms of conserved residues? (1 pt) *Tiebreaker 4*

43. Compare the two types of DNA repair involved with cleavage within the Cas9 system. (1 pt)
44. Which one is more accurate? (0.5 pts)

45. What are three other uses for the two types of DNA repair other than cleavage? (1 pt)

46. Describe how crRNA, tracrRNA, sgRNA, and gRNA are related to one another as well as their overall within the CRISPR/Cas9 system. (1 pt) *Tiebreaker 5*

47. The native PAM sequence for the commonly used SpyCas9 is (0.5 pts):

48. How many different main types (not including subtypes) of CRISPR systems exist? (0.5 pts)
    a. 2
    b. 4
    c. 6
    d. 8
    e. 18

49. What are the differences between type I and type II CRISPR systems? (0.5 pt)

50. Which type/system of CRISPR can Cas9 be found in? (0.5 pts)
    a. Type I
    b. Type II
    c. Type III
    d. All of the above
    e. None of the above

Section 3: APOBEC3A Cytidine Deaminase Protein

51. What role does H29 play within the protein? (1 pt)

52. What ion is present within the active site of the Cytidine Deaminase protein -- explain its significance? (1 pt)
53. Name the motif (present in the central nucleotides) bound at the active site along with the target deoxycytidine (dC0)? (1 pts)

54. What is one drawback of using cytidine base editors (explain why)? (1 pt)

55. Four different cytidine deaminase enzymes were evaluated for ssDNA deamination. Which of the following demonstrated the highest activity of deaminase? (1 pt) a. Human APOBEC3G
    b. Lamprey CDA1
    c. Rat APOBEC1
    d. Human AID

Congratulations on completing the University of Michigan 2021 Invitational Protein Modeling test!

If you have not done so already, please use this google form to turn in pictures of your prebuild model. This must be submitted before the timeslot ends -- links are at the top of the test.

If you have any questions or concerns pertaining to this event, please email tec.umichscioly@umich.edu, and we will try to get back to you as soon as we can.