C - Protein Modeling C 2020-2021 - BEARSO - 10-24-2020

Hello all competitors! This test contains Part 2 (Computer Exploration) and Part 3 (Written Exam) of the Protein Modeling event. If you have not done so already, please make sure you fill out the Protein Modeling Part 1 (Pre-build) form, following the format described in the form and on the BEARSO Events document. Best of luck on this test!

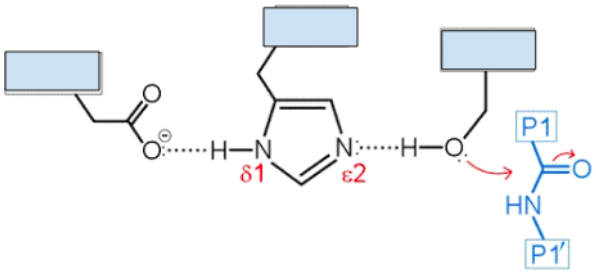
Try to format your answers the way we specify, but we will check for typos and other errors and try to give back any points the grader may have missed.

IMPORTANT: The Scilympiad platform will not penalize you for using the offline version of Jmol, so don't be afraid to open Jmol in a different window and leave the test window.

1. (4.00 pts)
PART 2: COMPUTER EXPLORATION

For this part, load the 2CHA PDB file using Jmol by clicking File > Get PDB and entering "2CHA" at the prompt. Alternatively, if this fails, you can download the PDB from the RCSB PDB website. If you use the RCSB PDB website, please do not look at anything on the page except for the "Download files" button.

Enzymes are studied using a diverse set of methods, one of which being structural biology, where scientists examine the structure of an active site and its chemical environment to understand the chemical mechanism. One of the most well understood enzymatic mechanisms is the catalytic triad, which is typically composed of an acidic, basic, and nucleophilic amino acid. In this question, we will study an enzyme that utilizes the catalytic triad.
Credit: Gonzaga University



First, open the 2CHA PDB file. This protein is composed up of multiple chains. How many chains are there?

6

2. (10.50 pts)
Name the non-redundant chains that make up an asymmetric unit. If Z, M, T, and A are the correct chains, enter your answer as AMTZ (alphabetical). For the rest of this test, until specified, we will only be working with the alphabetically first asymmetric unit.

ABC

3. (12.00 pts)
First, let's do some panning in Jmol. Find the residue number of each member of the catalytic triad, given that chymotrypsin typically uses histidine, serine, and aspartic acid in its catalytic mechanism. Type your answer in the format X#, where X is the one letter code of the amino acid and # is its number in the sequence. Answer in the order of acid, base, nucleophile.

D102 H57 S195

4. (8.00 pts)
While searching for the catalytic triad, I hope you noticed that there is another serine very close to it. Based on the reaction mechanism, explain structurally why this serine cannot participate in catalysis.

Expected Answer: The hydrogen on the serine hydroxyl group cannot be stabilized by the electronegative nitrogen because it is on the opposite side of the histidine compared to the correct serine. Thus, the oxygen cannot become nucleophilic enough to attack the substrate.

5. (8.00 pts)
Two of the secondary structures in this protein are alpha helices and beta sheets. Find the number of each in the asymmetric unit. First enter heclies, then enter sheets.

2 12

6. (16.00 pts)
The catalytic triad rests exclusively on one of the secondary structures in the protein. What secondary structure is this, and what are the pros and cons of this secondary structure in the context of catalysis?

Expected Answer: Loops (1) Loops are more flexible than helices and sheets which have a fairly rigid structure. Thus, they can orient themselves to accommodate different substrates, increasing the chance of catalysis upon contact with a substrate. (1.5) Flexibility also decreases specificity of the reaction mechanism. (1.5)

7. (8.00 pts)
IMPORTANT: AT THIS POINT, WE WILL BE WORKING WITH THE ENTIRE PDB FILE, NOT JUST THE ASYMMETRIC UNIT. There are two beta barrels in the asymmetric unit of the PDB: the chain B barrel and the (mostly) chain C barrel. To save you some time, note that part of chain B forms a barrel with all of chain C. If there are two boxes, answer using chain B first and chain C second. We will now do some fun counting/multiplication.

How many hydrogen bonds are in the asymmetric unit?

124

8. (8.00 pts) Are the barrels parallel or antiparallel? Is this more or less stable than the alternative? First write antiparallel/parallel, then write more/less. Please spell correctly!

antiparallel | more

9. (24.00 pts)
Calculate the average number of hydrogen bonds per strand for each barrel. First enter the barrel that contains only chain B, and then the barrel that contains both chains B and C. TWO DECIMAL PLACES.

4.83 | 5.17

10. (18.00 pts)
Given that hydrogen bond formation enthalpy is around -1.2 kcal/mol, and assuming that the only energetic interaction is the hydrogen bonding, what is the average enthalpy per beta strand residue in each barrel? Based on this analysis, which barrel is more thermodynamically stable? Answer in units of kcal/(Avogrado's number * atoms). Round to two decimal places. Enter chain B barrel first, then chain C barrel, and then B or C.

-0.83 | -0.87 | C

11. (8.00 pts) It turns out that the barrel cores are highly hydrophobic. List two of the hydrophobic amino acid types that you can find in the barrel cores.

Valine | Leucine

12. (8.00 pts) Besides proteins and water, there is one other type of small molecule in the PDB file. What is the name of this molecule? Enter the three letter abbreviation.

TSU

13. (8.00 pts)
One of these molecules participates in two water-coordinated hydrogen bonds. One of these is a sidechain-water-molecule hydrogen bond. Give the amino acid one letter code and position in the sequence that participates in the hydrogen bond.

H57

14. (8.00 pts)
You might have noticed that many backbone atoms only specify the alpha carbon, and the other atoms are missing from the structure. Infer the residue that participates in the other water-coordinate hydrogen bond and give its one-letter code and position. (We will accept two different residues as answers.)

(Mark **ALL** correct answers)

- [x] A) C42
- [x] B) F41

15. (8.00 pts)
The same molecule also hydrogen bonds to part of the protein backbone. Which residue does it bond to? Give your answer as the amino acid one letter code and the position in the sequence.

S195

16. (4.00 pts) Using the same procedure for extracting PDBs, open 5BVL.

What is the name of this super-secondary structure?

TIM Barrel

17. (7.00 pts) How many alpha helices are there?

8

18. (6.00 pts) How many beta sheets are there?

8

19. (4.00 pts) How many degrees of sequence symmetry does this protein exhibit?

4

20. (4.00 pts) Does the C-terminus correspond to a beta strand or an alpha helix?

beta strand

21. (4.00 pts) Is the barrel core hydrophobic or hydrophilic?

hydrophobic

22. (4.00 pts)
We define the “top” of the protein as the side where the beta strands are oriented. Based on the water molecules present in the PDB, is the top or bottom of the protein more hydrophilic?

bottom

23. (4.00 pts) An alternative way to create this protein structure is through domain fusion. Select the choice that is true.

- (●) A) This protein may have evolved from gene duplications
- ( ) B) The core region is highly hydrophobic and most important for catalytic activity
- ( ) C) The protein is not stable at physiological conditions due to disordered domains
- ( ) D) The protein is strictly structural in function

24. (4.00 pts) Does the N-terminus correspond to a beta strand or an alpha helix?

alpha helix

25. (0.00 pts)
We will now examine another TIM barrel called 1P1X. If possible, open this in another window because we will compare the two barrels. How many beta strands does 1P1X contain?

20

26. (0.00 pts) How many alpha helices does 1P1X contain?

22

27. (6.00 pts) Examine the side chains in the core of the protein. What kinds of network is crucial in stabilizing the barrel?

Expected Answer: salt bridge

28. (4.00 pts) Compare this to 5BVL. Which energetic effect stabilizes the core?

Expected Answer: hydrophobic effect

29. (3.00 pts)
PART 3: WRITTEN EXAM

SECTION: Cytidine Deaminase

You are refining the structure of a protein and end up with R-factor/R-free values of 0.224/0.538. This value indicates a (1) quality protein structure because R-free is (2) the R-factor. The diffraction pattern of the calculated model (3) the experimentally observed diffraction pattern. Type the three words in order.

Expected Answer: You are refining the structure of a protein and end up with R-factor/R-free values of 0.224/0.538. This value indicates a (1) quality protein structure because R-free is (2) the R-factor. The diffraction pattern of the calculated model (3) the experimentally observed diffraction pattern. Type the three words in order.

30. (3.00 pts)

Now that you know how to interpret R-values in crystallography (I hope), you read the Schiffer paper that reports the structure of APOBEC3A. You notice that the R-factor/R-free is reported as (4, write your answer as R-factor/R-free). This indicates that the model is a (5) quality protein structure because R-free is (6) the R-factor. Type the three answers in order.

Expected Answer: 0.177/0.225 high similar to

31. (1.00 pts) Residue Y130 contributes to dC0 positioning through a T-shaped interaction with the pyrimidine ring. What type of interaction is this?

Expected Answer: pi-pi

32. (6.00 pts) Hypothesize why N57 is highly conserved in APOBEC protein domains. (3 reasons please.)

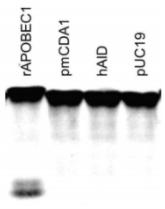
Expected Answer: N57 sidechain determines ssDNA directionality by hydrogen bonding to O3' of dC0. N57 sidechain hydrogen bonds with and orients T31, which hydrogen bonds with the pi-orbital cloud of the dC0. N57 sidechain packs against dC0 and H70 which orients them to stabilize the pentose plane and coordinate zinc. (This counts as two reasons.)

33. (6.00 pts) If APOBEC3A were to operate on RNA, a conformational change would have to occur in the active site of the enzyme. Explain why.

Expected Answer: Steric clash between 2'-OH and H70 would manifest, requiring a rearrangement of the active site for RNA modification.

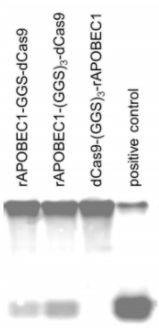
34. (0.00 pts) SECTION: Base Editor Design

There are roughly two types of bands on this PAGE gel displayed below. Explain what is in the band on the right and what is in the band on the left.



Expected Answer: left: cleaved U-containing products right: C-containing substrates

35. (2.00 pts)
The bands in the below diagram diagram are the same as the bands in the previous question. Explain the difference between rAPOBEC1-(GGS)3-dCas9 and dCas9-(GGS)3-rAPOBEC1, and predict which protein they would use to construct the base editor with.



Expected Answer: The dCas9 is appended to different termini (C and N termini of rAPOBEC1) They should use rAPOBEC1-(GGS)3-dCas9 because it has greater deaminase activity, as indicated by the darker band on the left.

36. (2.00 pts) How many residues is the XTEN linker used in BE1? What was the point of the experiments that varied the length of the XTEN linker?

Expected Answer: 16 residues The purpose of the experiments was to determine the nucleotide range that the deaminase could perform deamination on based on the length of the linker.

37. (2.00 pts) Why was UGI appended to BE2?

Expected Answer: UDG catalyzes removal of U from DNA and initiates base-excision repair, and UGI inhibits UDG activity. Thus, UGI was added to increase editing efficiency of the base editor by inhibiting the effect of UDG.

38. (2.00 pts)
SECTION: CRISPR-Cas12b

The CRISPR systems you have studied typically directly edit the genetic material of a cell using a variety of different techniques, some more effective than others. However, the ability to target a certain sequence in a cell's genome extends far beyond simply editing that sequence. You attach a transcriptional effector molecule to dCas9 protein. What effect does this modified dCas9 have on the gene of interest?

Expected Answer: The protein encoded by this gene will increase dramatically in the cell.

39. (2.00 pts) Thinking about this in the reverse fashion: how can dCas9 be used to prevent the expression of a protein?

Expected Answer: dCas9 can be engineered to bind promoter regions of a gene or chemicals that inhibit transcription.

40. (3.00 pts)
A newly discovered Cas protein, called Cas12b, is finding great uses in plant genome manipulation. This Cas protein's PAM is VTTV. Name three different valid PAMs that Cas12b can bind using specific nucleotides.

Expected Answer: any TTTV

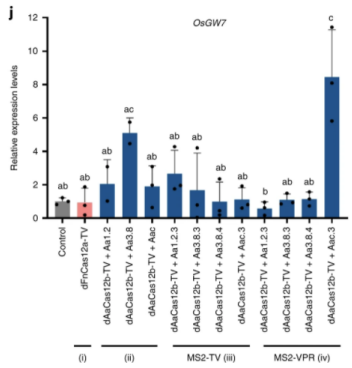
41. (1.00 pts) The Cas12b protein (also called C2c1) lacks a domain that is found in Cas9 that is used to recognize the PAM. What is this domain called?

Expected Answer: PAM Interacting domain

42. (1.00 pts) What does the title of the graph, "OsGW7," refer to?

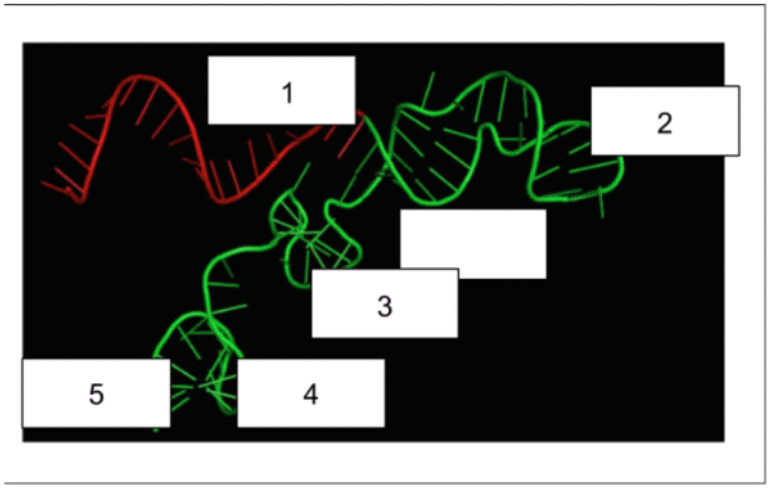
Expected Answer: the gene that is being expressed

43. (2.00 pts)
In this experiment, Cas12b was mutated to remove its catalytic function and instead was used as a transcription factor. Based on the graph, did dAaCas12b successfully bind the gene of interest?



Expected Answer: yes because the expression level increases in the experimental trial

Labeling!



44. (0.50 pts) 1.

Expected Answer: Target complementary region

45. (0.50 pts) 2.

tetraloop

46. (0.50 pts) 3. _____ _____ __ (three word answer, enter exact).

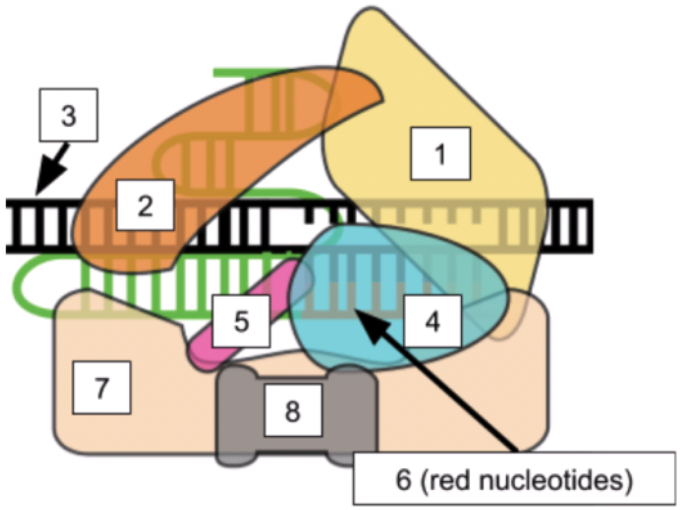
stem	loop	1

47. (0.50 pts) 4. _____ _____ __ (three word answer, enter exact).

stem	loop	2

48. (0.50 pts) 5. _____ _____ __ (three word answer, enter exact).

stem	loop	3



49. (0.50 pts) 1. (enter domain name)

RuvC

50. (0.50 pts) 2. (enter domain name)

PAM interacting

51. (0.50 pts) 3. (abbreviate)

DNA

52. (0.50 pts) 4. (enter domain name)

HNH

53. (0.50 pts) 5. (two words, enter in one blank with a dash separating -- no spaces!)

bridge-helix

54. (0.50 pts) 6.

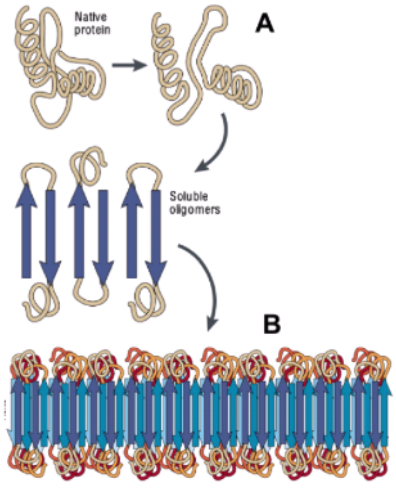
Expected Answer: Target complementarity region

55. (0.50 pts) 7. (enter domain name)

REC1

Part A: Morpheeins (Wikipedia - "Not to be confused with Morphine")

While conformational changes in morpheeins are crucial to function, mutations can also result in clinical diseases such as Alzheimer's disease and Huntington's disease. Fill in the blanks of the following model of neurodegenerative diseases.



56. (0.00 pts) A:

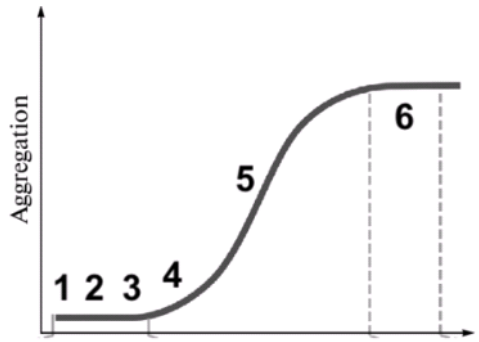
Expected Answer: accept: misfolded protein or equivalent

57. (0.00 pts) B:

Expected Answer: Accept fibril or amyloid fibril

Fill in the blanks in the following model of amyloid fibril formation using the word bank below. Type ONLY the letter (A, B, C, D, E, F) in the subsequent fill in the blank questions.

A - Protofibril	**B-** Oligomer	**C-** Amyloid Fibril	**D-** Misfolded monomer	**E-** Native Protein	**F-** Dimer



58. (0.50 pts) 1. ____

E

59. (0.50 pts) 2. ____

D

60. (0.50 pts) 3. ____

F

61. (0.50 pts) 4. ____

B

62. (0.50 pts) 5. ____

A

63. (0.50 pts) 6. ____

C

Morpheeins

The standard model of allosteric regulation of proteins considers an equilibrium of among protein structures of constant oligomeric multiplicity, with classic structures highlighting a dramatic change in the structure and function of the individual subunits. A familiar example may be the 'T state' and 'R state' of hemoglobin -- regardless of the current state of the system, the multiplicity is tetrameric. The morpheein model contrasts in that the multiplicity of the quaternary state could change following a structural change induced by allosteric regulation.

A well characterized morpheein is the enzyme porphobilinogen synthase, **PBGS**, which has a functional distinction in the level of activity of different oligomers. PBGS will bind two molecules of ALA and catalyze a condensation reaction. To further investigate the morpheein model, several experiments are performed. Use the data presented and background knowledge about biochemical techniques to provide brief responses.

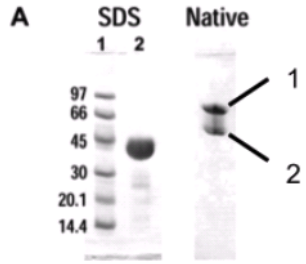


Figure 1: A) SDS-PAGE and Native-PAGE of purified PBGS protein

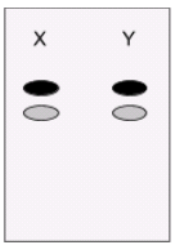
64. (1.00 pts)
Question 1: In Native-PAGE, the protein of interest is in the _____ state versus the _____ state in SDS-PAGE, and the mobility is dependent on the charge-to-mass ratio and the _____ and _____ of the protein. Separate each answer with a comma.

Expected Answer: Folded, Unfolded, size / shape

65. (2.00 pts)
Question 2: Figure 1A gives credibility to several hypotheses of the morpheein model. Discuss how the results of the figure support the morpheein model. In your response, include why a native gel was necessary to show these results.

Expected Answer: Presence of multiple oligomeric states. SDS PAGE gel is denaturing and demonstrates that subunits of PBGS have the same molecular mass. Thus, from the native PAGE gel, there must exist multiple oligomeric states (1,2) which is consistent with the morpheein model. The native gel is not denaturing and will allow for non-covalent subunit interactions in the oligomeric protein to persist.

66. (3.00 pts)
Question 3: The researchers purify bands 1 and 2 in the native gel into separate vials (with a stable buffer). Unfortunately, one absentminded researcher loses the labels and is forced to figure out which is which. After running a native PAGE, the following results are shown, where X and Y correspond to the unknown samples.

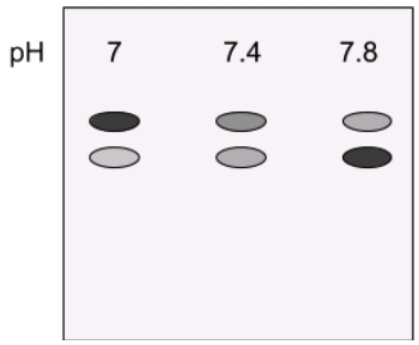


Explain these results and if it's possible for the researcher to determine the original labelling. If so, propose a series of experiments the researcher could use.

Expected Answer: Oligomeric states have some equilibrium and conversion between different oligomeric states is possible, resulting in the gel. It is not possible to determine the original labelling at current state since a new equilibrium has been reached for this buffer and the two vials are indistinguishable. No credit if experiment proposed.

67. (4.00 pts)

Question 4: Further investigation reveals that PBGS enzymatic activity in some conditions does not fit well with the standard Michaelis-Menten equation. After running purified PBGS with a native PAGE under different pH conditions, the following data is shown.



You notice that at neutral pH, the protein has kinetics that fit well with the Michaelis-Menten model, but not at higher pH. You measure the rate of the reaction with 10 mM ALA ([S] >> [E]) and find the reaction rate at different pH conditions.

7.0	7.4	7.8
48.0 uM / min	20.2 uM / min	5 uM/ min

Predict which band from Figure 1A has a higher V_max. Discuss how the results above support this and how PBGS is likely to affect enzyme activity. Discuss how this is consistent with the morpheein model and your response to question 2.

Expected Answer: pH dependent oligomeric shifts; band 1 is the oligomer with high activity; band 2 is the oligomer with low activity; consistent with morpheein model due to oligomeric “switching” at a basic pH. Do not accept denaturation -- would not be notable at this pH range and is not supported on the native PAGE gel.

Kinetics

Question 1: While studying the enzyme MOOP, you realize that certain treatments can affect two important kinetic properties: V_max and K_m from the Michaelis-Menten model. Your PI asks you to classify the following inhibitors as competitive, uncompetitive, noncompetitive, or mixed based on their effects on the apparent V_max and apparent K_m of binding.
Note that u = micro- prefix.

Original Enzyme: V_max = 2.3 uM / min ; K_m = 55 uM

1. V_max = 2.3 uM / min ; K_m = 85 uM
2. V_max = 1.3 uM / min ; K_m = 29.9 uM
3. V_max = 2.1 uM / min ; K_m = 65 uM
4. V_max = 0.6 uM / min ; K_m = 55 uM
5. V_max = 0.3 uM / min ; K_m = 10 uM
6. V_max = 1.9 uM / min ; K_m = 43.7 uM

68. (1.00 pts) 1.

- (●) A) Competitive
- ( ) B) Uncompetitive
- ( ) C) Noncompetitive
- ( ) D) Mixed

69. (1.00 pts) 2.

- ( ) A) Competitive
- (●) B) Uncompetitive
- ( ) C) Noncompetitive
- ( ) D) Mixed

70. (1.00 pts) 3.

- ( ) A) Competitive
- ( ) B) Uncompetitive
- ( ) C) Noncompetitive
- (●) D) Mixed

71. (1.00 pts) 4.

- ( ) A) Competitive
- ( ) B) Uncompetitive
- (●) C) Noncompetitive
- ( ) D) Mixed

72. (1.00 pts) 5.

- ( ) A) Competitive
- ( ) B) Uncompetitive
- ( ) C) Noncompetitive
- (●) D) Mixed

73. (1.00 pts) 6.

- ( ) A) Competitive
- (●) B) Uncompetitive
- ( ) C) Noncompetitive
- ( ) D) Mixed

Question 2: In addition, match the following equations of Michaelis-Menten binding with competition to the type of inhibition along with a brief (1-2 sentence) explanation of how it is consistent with the expected changes in V_max and K_m for each inhibitor.

A

$$v = V_1 \frac{[S]}{[S] + K_1\left(1 + \frac{[I]}{K_i}\right)}$$

B

$$v = V_1 \frac{[S]}{([S] + K_1)\left(1 + \frac{[I]}{K_i}\right)}$$

C

$$v = V_1 \frac{[S]}{[S]\left(1 + \frac{[I]}{\alpha K_i}\right) + K_1\left(1 + \frac{[I]}{K_i}\right)}$$

74. (1.50 pts) A.

Expected Answer: competitive; some explanation of V_max vs K_m suffices

75. (1.50 pts) B.

Expected Answer: noncompetitive -- some sentence explanation

76. (1.50 pts) C.

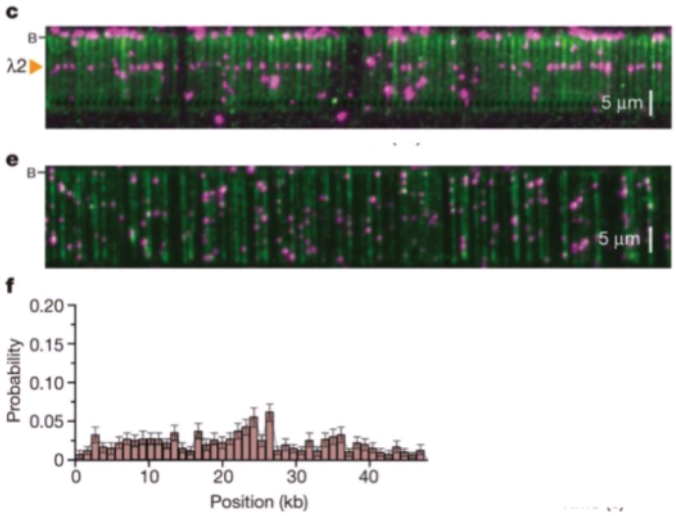
Expected Answer: mixed -- some explanation

CRISPR

CRISPR / Cas9 systems bind DNA and recognize a specific cleavage site. The schematics and data below represent the results of an experiment conducted in order to better understand how Cas9 binds DNA.

The researchers tether λ-DNA substrates (48k pb) and examine binding of purified *S. pyogenes* Cas9 with a C-terminal FLAG tag that allows for fluorescent labeling. Generated guide RNA had complementarity to one 20-bp site inside of the λ-DNA. Using total internal reflection microscopy, the researchers are able to visualize binding of single Cas9 molecules on DNA substrate. The following results are reported:

Note that figure C and E/F represent the two conditions of Cas9 binding tested in the assay. Both experiments are performed *in vitro* with identical physical conditions (temperature, pH, etc).



Adapted from Sternberg et. al. 2014

77. (1.00 pts)

Question 1: To prevent Cas9 from creating double stranded breaks in the λ-DNA substrate, the researchers generated an inactivated dCas9 protein that has a mutation in the _____ domain (non-target strand) and _____ domain (target strand).

RuvC | HNH

78. (1.00 pts)

Question 1.5: The two mutations generated, in general, are _____ (non-target strand) and _____ (target strand). Mutations should be in the form of (AA-#-AA ex: F36T) with no spaces. Fill in the blank.

D10A | H840A

79. (3.00 pts) **Question 2:** The dCas9 protein retains DNA binding activity. Identify all of the following applications where dCas9 would be applicable.

(Mark **ALL** correct answers)

- [x] A) Targeted base editing with cytidine deaminase
- [x] B) Transcriptional repression *in vivo*
- [x] C) Recruitment of transcriptional activators *in vivo*
- [ ] D) Targeted degradation of mRNA transcripts
- [ ] E) Targeted binding of mRNA transcripts to prevent ribosomal initiation

80. (3.00 pts)

Question 3: Figure C and E show fluorescent microscopy images of the experiments performed. Plot E represents the form _____-Cas9 in contrast to Cas9 with _____ bound. Explain why the first form of Cas9 leads to the results in figure E.

Expected Answer: apo-Cas9; RNA: or RNA bound -- absence of guide RNA results in nonspecific binding to any PAM sequence

81. (3.00 pts)
Question 4: AcrIIA4 is a competitive inhibitor of Cas9 that competes for binding to the PAM recognition site. You note that Cas9 binding to AcrIIA4 has a lower K_d than Cas9 binding to DNA with the proper PAM site. Predict how AcrIIA4 would change the results observed in Figure C and Figure E and offer an explanation.

Expected Answer: Little to no binding = no fluorescent spots / Cas9 tagged bound-- competitive inhibitor with higher K_d would be expected to essentially prevent binding

82. (2.00 pts)
Question 5: You discover an additional inhibitor of Cas9 and run the same set of experiments as the researchers to discover that both experiments result in a similar distribution to figure E/F. Identify all of the following statements that describe hypotheses consistent with these results.

(Mark **ALL** correct answers)

- [ ] A) The inhibitor could disrupt Cas9 binding to the PAM and thus disrupts DNA binding
- [x] B) The inhibitor could disrupt Cas9 binding to the guide RNA and thus prevent specific binding
- [ ] C) The probability distribution of Cas9 binding with the inhibitor is unlikely to match figure F when guide RNA is introduced *in vitro*
- [ ] D) The inhibitor could disrupt Cas9 binding by denaturing the Cas9 protein to the point where DNA binding is random.
- [ ] E) The inhibitor could disrupt Cas9 binding by "locking" the DNA in the double stranded configuration

83. (2.00 pts)
Question 6: You discover a novel Cas9 protein with the PAM sequence 5' NGA 3' Approximately how many PAM sites would you expect on the target strand of a 40kb dsDNA viral genome with equal amounts of each nucleotide base. Provide an explanation / work for partial credit.

Expected Answer: ~2500

84. (2.00 pts)
Question 7: Six different sgRNAs are generated that all have perfect homology to different 20 bp segments in the λ-DNA substrate. Interestingly, only 3 of the generated sgRNAs display results similar to figure C after experimentation is conducted. Provide a brief explanation for why this might occur.

Expected Answer: Not located near a PAM site

85. (1.00 pts) Cas9 is most commonly associated with which CRISPR-Cas system?

- ○ A) Type I

- [x] B) Type II
- [ ] C) Type III
- [ ] D) Type IV

86. (1.00 pts) Archaea typically use which CRISPR-Cas system?

- [ ] A) Type I
- [ ] B) Type II
- [x] C) Type III
- [ ] D) Type IV

87. (2.00 pts) Which of the following polymers is expected to have the longest persistence length (assuming the same number of monomers for each polymer)

- [ ] A) poly(dT)
- [x] B) poly(dA)
- [ ] C) poly(proline)
- [ ] D) poly(glycine)

88. (1.00 pts) Which of the following polymers has the lowest turn penalty?

- [ ] A) poly(proline)
- [x] B) poly(glycine)
- [ ] C) poly(tryptophan)
- [ ] D) poly(glutamine)

89. (1.00 pts) poly(proline) has the strongest preference for which of the following conformations?

- [x] A) Trans isomers of its peptide bonds
- [ ] B) Cis isomers of its peptide bonds
- [ ] C) An extended conformation

90. (1.00 pts)
An important part of protein and peptide interactions is the interaction with lipid bilayers found at cell walls. Which of the following peptide sequences would you expect to have the greatest cellular penetration? Take note of the D and L amino acid forms when responding.

- [ ] A) d-(Lys-Lys-Lys-Lys-Trp-Lys-Lys)
- [ ] B) l-(Lys-Lys-Gln-Lys-Lys-Lys-Lys)
- [ ] C) l-(Arg-Arg-Arg-Arg-Ala-Arg-Arg)
- [x] D) d-(Arg-Arg-Arg-Arg-Arg-Arg-Arg)

91. (2.00 pts)
Typically, in DNA, A is matched with T (ie. A-T), and C is matched with G (ie. C-G). Mismatches are often disruptive to the DNA structure. Which of the following mismatches are usually the **least** disruptive to stability?

- [x] A) G-A
- [ ] B) C-C

C) G-G

D) A-A

92. (2.00 pts)
Typically, in DNA, A is matched with T (ie. A-T), and C is matched with G (ie. C-G). Mismatches are often disruptive to the DNA structure. Which of the following mismatches are typically the **most** disruptive to stability?

A) G-A

B) C-C

C) G-G

D) A-A

93. (3.00 pts)
Mutating residues on a protein could cause the protein to misfold, or change the stability of the protein. Say you are using yeast surface display expressing the protein IL-11R with a single amino acid mutation, and would like to assess the stability of this mutant. You also have access to yeast expressing wild-type IL-11R, as well as monoclonal antibodies targeting a conformational epitope on the protein. Binding experiments in your lab are typically carried out with inducing 1 million yeast, with bind carried out at room temperature, assessed by flow cytometry. Which of the variables would you alter to most directly assess the stability of the mutant protein, while also preserving the viability of the yeast?

A) Number of yeast used in the experiments

B) Replacing the monoclonal antibody used in the binding assessment with polyclonal antibodies

C) Replacing the monoclonal antibody used in the binding assessment with one targeting a linear epitope on IL-11R

D) Varying the temperature of the yeast while otherwise repeating the same binding experiment.

E) Replacing the yeast media with 500mM NaCl during binding experiment.

94. (1.00 pts) The Watson-Crick base pair scheme for an A-T base pair includes:

A) A hydrogen bond between the 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

95. (1.00 pts) Which nucleobase is known for its tendency to form square planar quartet structures in sequences rich in that nucleobase?

A) T

B) A

C) W

D) G

E) U

F) C

96. (2.00 pts)
Proteins have different optimal structures depending on temperature. Consider the following plot on Figure 1. Let's say this plot represents the potential energy of a given protein as it adopts different conformations.

For the purposes of the problem, assume that all the energy wells shown in the figure have the same area.

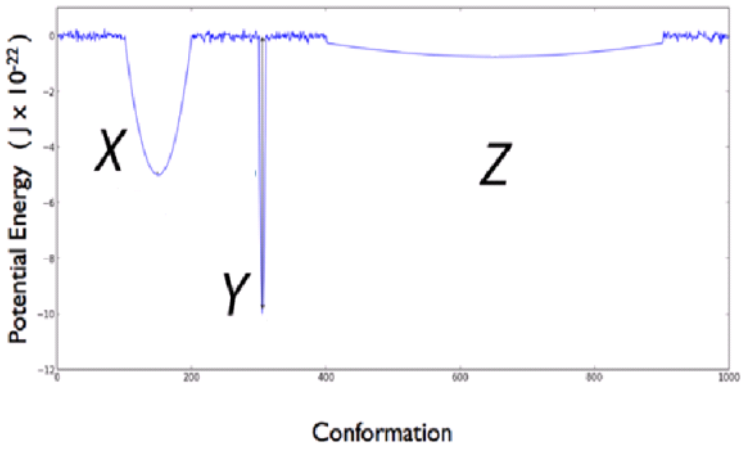


Figure 1: Potential Energy vs. Conformation

Which conformational macrostate is preferred at T=0K? (Hint: In these regimes, we would approach 0K from the positive direction)

- A) Macrostate X
- B) Macrostate Y
- C) Macrostate Z

97. (2.00 pts)

Proteins have different optimal structures depending on temperature. Consider the following plot on Figure 1. Let's say this plot represents the potential energy of a given protein as it adopts different conformations.

For the purposes of the problem, assume that all the energy wells shown in the figure have the same area.

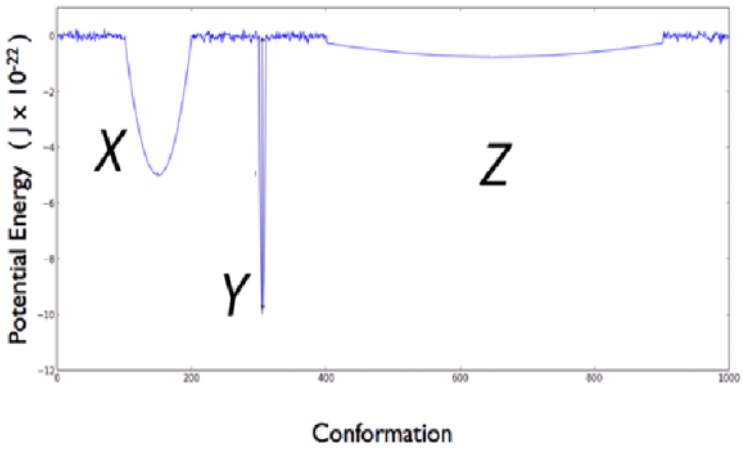


Figure 1: Potential Energy vs. Conformation

Which conformational macrostate is preferred at high temperatures (mathematically put, as T approaches infinity?)

- A) Macrostate X
- B) Macrostate Y
- C) Macrostate Z

Biochemistry

98. (0.50 pts) Which of the following is a difference between DNA and RNA?

- A) Synthesis from nucleotide triphosphates
- B) Ability to be degraded by exonucleases
- C) Presence of a 2' OH
- D) Formation of secondary structures
- E) Ability to form hairpins

TRUE FALSE:

99. (0.50 pts) Disulfide bonds occur between two cysteine residues and can stabilize protein structure

True False

100. (0.50 pts) Attractive electrostatic interactions can occur between two charged amino acid side chains when both are positive or both are negative

True False

101. (0.50 pts) Hydrogen bonding is possible only when a hydrogen atom is bonded to oxygen or nitrogen

True False

102. (0.50 pts) A genetic mutation that affects the primary structure of a polypeptide would alter the amino acid sequence of the polypeptide

True False

103. (0.50 pts) Hemoglobin, which has 4 subunits, is an example of a protein with quaternary structure

True False

104. (0.50 pts) Alpha helices and beta sheets are examples of structures held together by hydrogen bonding

True False

105. (0.50 pts) Hydrophobic amino acids are likely to be found away from the external surface of the protein that is exposed to water

True False

106. (0.50 pts) A protein folded in the native state would typically have a higher Gibbs free energy than the unfolded conformation

True False

107. (0.50 pts) Excessive temperature, extreme pH, and high solute concentration can all denature a protein

(●) True ( ) False

108. (0.50 pts) Large proteins can have masses of upwards of 100 kDa (kilo-daltons)

(●) True ( ) False

A scientist identifies a self-replicating RNA molecule that has ribozyme activity. Identify the following statements as True or False:

109. (0.50 pts) Ribosomal RNA is another example of a ribozyme

(●) True ( ) False

110. (0.50 pts) The self replicating RNA violates the Central Dogma

(●) True ( ) False

111. (0.50 pts) This molecule would likely have higher stability in physiological conditions than DNA

( ) True (●) False

112. (0.50 pts) The findings violate the principle of minimal entropy

( ) True (●) False

113. (0.50 pts) A self-replicating RNA molecule lends support to the RNA world hypothesis

(●) True ( ) False

Match the amino acid to the properties listed in the questions below. Enter one character into each blank in the following questions.

a) Leucine
b) Glycine
c) Proline
d) Arginine
e) Alanine
f) Aspartate
g) Histidine
h) Serine
i) Tyrosine
j) Valine
k) Serine
l) Phenylalanine

114. (0.50 pts) Only achiral amino acid

B

115. (0.50 pts) Aromatic and hydrophobic, frequently found in core region

L

116. (0.50 pts) Found in beta-turns, imino acid, helix-breaker

C

117. (0.50 pts) Positively charged at physiological pH, can shift pKa's

G

118. (0.50 pts) Aromatic, frequently phosphorylated as part of regulatory processes

I

119. (0.50 pts) Polar, often found in the active site of an enzyme and facilitates proton-transfer reactions

H

120. (0.50 pts) Side chain is a methyl group

E

121. (0.50 pts) Highest pKa of side chain (in list of amino acids)

D

122. (0.50 pts) Lowest pKa of side chain (in list of amino acids)

F

123. (1.00 pts) Synthesized via reductive amination of pyruvate

E

124. (1.00 pts) Which of the following is TRUE about gel electrophoresis? Select all that apply.

(Mark **ALL** correct answers)

- [x] A) Gel electrophoresis can be used to separate DNA molecules based on size
- [ ] B) Larger DNA molecules will migrate faster than slower DNA molecules
- [x] C) The presence of a DNA ladder or molecular-weight marker in one well of the gell will allow for rough estimation of sample length
- [x] D) DNA will migrate toward the positive electrode
- [ ] E) DNA will migrate toward the negative electrode

125. (1.00 pts) Which of the following dsDNA strands would have the highest melting point, otherwise denoted T_m?

- ○ A) 5' TTTTTAAAAA 3'
  3' AAAAATTTTT 5'
- ○ B) 5' TTAAAGCGAA 3'
  3' AATTTCGCTT 5'
- ◉ C) 5' GGCCACAGA 3'
  3' CCGGTGTCT 5'
- ○ D) 5' TTCAAAAGA 3'
  3' AAGTTTTCT 5'

Your friend Rosalind is an expert at analyzing x-ray diffraction patterns and tells you that there are several distinct forms of DNA in different conditions. Match the conditions below to the expected properties of the form. Note that each condition represents a distinct form of DNA. Write only the letter that corresponds in each blank in the following questions.

a. Aqueous solution at physiological pH with low solute concentration
b. Dehydrating conditions expected prior to crystal formation
c. DNA with high negative supercoiling in an aqueous solution at physiological pH with high salt concentration

126. (1.00 pts) Deep and narrow major groove with a wide and shallow minor groove

B

127. (1.00 pts) Left handed helical structure (maintains anti-parallel strands)

C

128. (1.00 pts) Broadest and most compressed form of DNA

B

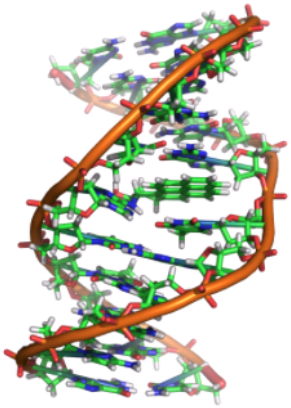
129. (1.00 pts) Anti : pyrimidine; syn: purine glycosyl angle

C

130. (1.00 pts) Right handed helix with exposed major / minor groove that can be recognized by HLH transcription factors

A

131. (2.50 pts) In an unfortunate turn of events, the DNA in one of your favorite cells was altered in the manner below. Select all of the following choices that are TRUE.



(Mark **ALL** correct answers)

- [ ] A) The DNA strand would have entropic-flexibility that is consistent with polymer-physics models of bending, such as the worm-like chain model and.
- [x] B) The disruption of pi stacking interactions likely reduces stability of the DNA
- [x] C) The alteration could represent the action of an intercalating agent
- [x] D) Repair processes would rely on nucleotide excision repair instead of base excision repair
- [ ] E) The repair pathway would result in the individual excision of the affected base, forming an abasic site in the process

132. (2.50 pts) Select all of the following that represent properties important to the circularization of DNA? Select all that apply.

(Mark **ALL** correct answers)

- [x] A) Axial stiffness
- [x] B) Torsional stiffness
- [x] C) DNA length
- [x] D) Integral number of turns of the DNA helix
- [ ] E) 5' and 3' base pair identity

133. (3.00 pts) Which of the following polymers is expected to have the longest persistence length (assuming the same number of monomers for each polymer)

- ( ) A) poly(dT)
- (•) B) poly(dA)
- ( ) C) poly(proline)
- ( ) D) poly(glycine)

134. (3.00 pts) Which of the following polymers (assuming the polymers have the same number of amino acids) is the most rigid?

- (•) A) poly(proline)
- ( ) B) poly(glycine)
- ( ) C) poly(tryptophan)
- ( ) D) poly(glutamine)

135. (2.00 pts) Which of the following polymers has the lowest turn penalty?

A) poly(proline)

B) poly(glycine)

C) poly(tryptophan)

D) poly(glutamine)

136. (2.00 pts) poly(proline) has the strongest preference for which of the following conformations?

A) Trans isomers of its peptide bonds

B) Cis isomers of its peptide bonds

C) An extended conformation

137. (2.50 pts)
An important part of protein and peptide interactions is the interaction with lipid bilayers found at cell walls. Which of the following peptide sequences would you expect to have the greatest cellular penetration? Take note of the D and L amino acid forms when responding.

A) d-(Lys-Lys-Lys-Lys-Trp-Lys-Lys)

B) l-(Lys-Lys-Gln-Lys-Lys-Lys-Lys)

C) l-(Arg-Arg-Arg-Arg-Ala-Arg-Arg)

D) d-(Arg-Arg-Arg-Arg-Arg-Arg-Arg)

138. (1.50 pts)
Typically, in DNA, A is matched with T (ie. A-T), and C is matched with G (ie. C-G). Mismatches are often disruptive to the DNA structure. Which of the following mismatches are usually the **least** disruptive to stability?

A) G-A

B) C-C

C) G-G

D) A-A

139. (1.50 pts)
Typically, in DNA, A is matched with T (ie. A-T), and C is matched with G (ie. C-G). Mismatches are often disruptive to the DNA structure. Which of the following mismatches are typically the **most** disruptive to stability?

A) G-A

B) C-C

C) G-G

D) A-A

140. (3.00 pts)
Mutating residues on a protein could cause the protein to misfold, or change the stability of the protein. Say you are using yeast surface display expressing the protein IL-11R with a single amino acid mutation, and would like to assess the stability of this mutant. You also have access to yeast expressing wild-type IL-11R, as well as monoclonal antibodies targeting a conformational epitope on the protein. Binding experiments in your lab are typically carried out with inducing 1 million yeast, with bind carried out at room temperature, assessed by flow cytometry. Which of the variables would you alter to most directly assess the stability of the mutant protein, while also preserving the viability of the yeast?

A) Number of yeast used in the experiments

B) Replacing the monoclonal antibody used in the binding assessment with polyclonal antibodies

C) Replacing the monoclonal antibody used in the binding assessment with one targeting a linear epitope on IL-11R

D) Varying the temperature of the yeast during the experiments.

E) Replacing the yeast media with 500mM NaCl during binding experiment.

141. (2.50 pts) The Watson-Crick base pair scheme for an A-T base pair includes:

A) A hydrogen bond between the 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

142. (2.00 pts) Which nucleobase is known for its tendency to form square planar quartet structures in sequences rich in that nucleobase?

A) A
B) T
C) C
D) G
E) U

Proteins have different optimal structures depending on temperature. Consider the following plot on Figure 1. Let's say this plot represents the potential energy of a given protein as it adopts different conformations.
For the purposes of the problem, assume that all the energy wells shown in the figure have the same area.

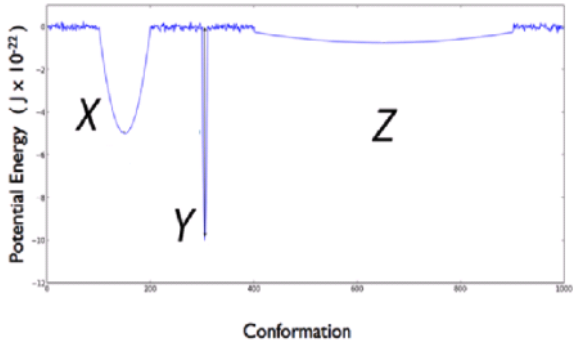


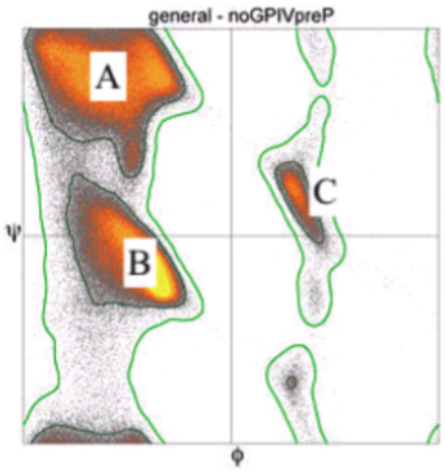
Figure 1: Potential Energy vs. Conformation

143. (2.00 pts)
Which conformational macrostate is preferred at T=0K? (Hint: In these regimes, we would approach 0K from the positive direction). Answer with a single character.

Y

144. (2.00 pts) Which conformational macrostate is preferred at high temperatures (mathematically put, as T approaches infinity?). Answer with a single character.

Z



Use the following bank of choices to answer questions (write the letter corresponding to the correct choice in the blank)

a. Beta-sheet
b. Left handed alpha-helix
c. Right handed alpha-helix
d. poly(proline)
e. poly(glycine)

145. (1.50 pts) Which of the above choices corresponds to **A** in the Ramachandran plot above?

A

146. (1.50 pts) Which of the above choices corresponds to **B** in the Ramachandran plot above?

C

147. (1.50 pts) Which of the above choices corresponds to **C** in the Ramachandran plot above?

B

148. (1.50 pts) Contains only cis-isomers of peptide bonds

D

149. (1.50 pts)
A researcher is investigating a thermophilic bacteria and finds, interestingly, that the bacteria does not have a higher than expected amount of heat shock proteins. Further analysis of the proteome reveals enrichment in leucine, phenylalanine, and isoleucine. Select all of the following that are TRUE.

(Mark **ALL** correct answers)

- [ ] A) Hydrophobic amino acids are expected to "insulate" protein-protein interaction from the high energy water molecules in the environment
- [ ] B) These amino acids are large and restrict the dihedral angle values of phi and psi to a stable conformation
- [x] C) Increased hydrophobic sheltering would contribute to the hydrophobic effect and stabilizing the protein at the high temperature
- [ ] D) Non-standard imino acids could facilitate a similar function in an alternative species
- [ ] E) Enrichment in these amino acids would also be expected in proteins without globular structure, such as a high temperature analog of collagen.

150. (2.50 pts)
You are interested in purifying a novel protein, moopase, with enzymatic activity from cell lysate. From bioinformatic analysis, you know that your protein has an isoelectric point, or pI of 6.4. Your professor instructs you to use a variety of techniques to do so. Select all of the following choices that are TRUE about protein purification techniques and your protein of interest.

(Mark **ALL** correct answers)

- [x] A) At a buffer pH of 7.4 you would expect your protein to have a negative charge and bind to a anion-exchange column as part of column chromatography
- [ ] B) At a buffer pH of 7.4 you would expect your protein to have a positive charge and bind to a cation-exchange column as part of column chromatography
- [x] C) After purifying your protein, you mix it up with two other proteins of pI 7.3 and pI 7.0. At a buffer pH of 6, you would expect your protein to elute FIRST with a cation-exchange column.
- [ ] D) Given your protein has a size of 60 kDa, a size exclusion column that can fractionate between 3 kDA and 50 kDa would be appropriate for ensuring your protein elutes LAST.
- [x] E) Attaching a His-tag to the C-terminus of your protein could allow for purification with a column with nickel ions affixed to the resin and elution by imidazole.

The Michaelis-Menten model of enzyme kinetics is the following relates the rate of product formation to the concentration of a substrate. You're analyzing an enzyme, E, that catalyzes a reaction with a single substrate, converting A → B. Options below represent scenarios that have True / False answers. Denote which are true and false in the questions below.

151. (1.50 pts)
At low substrate concentration, [A] << K_m, the reaction rate is accurately modeled by first order kinetics and will have a linear relationship with substrate concentration, [A].

- (●) True
- ( ) False

152. (1.50 pts) As [A] approaches infinity, based on the Michaelis-Menten model, the second derivative of product concentration with respect to time (d^2[B] / dt^2) will approach 0.

- (●) True
- ( ) False

153. (1.50 pts) Immediately after substrate addition, pre-steady state, the rate of change of the concentration of the enzyme substrate complex (d[EA]/ dt) is strictly decreasing.

- (●) True
- ( ) False

154. (1.50 pts)
The Michaelis-Menten model relies on the assumption that the reaction is irreversible, thus many cellular reactions with a Gibbs free energy change close to 0 (between 0 and -1 kJ) are excluded.

- (●) True
- ( ) False

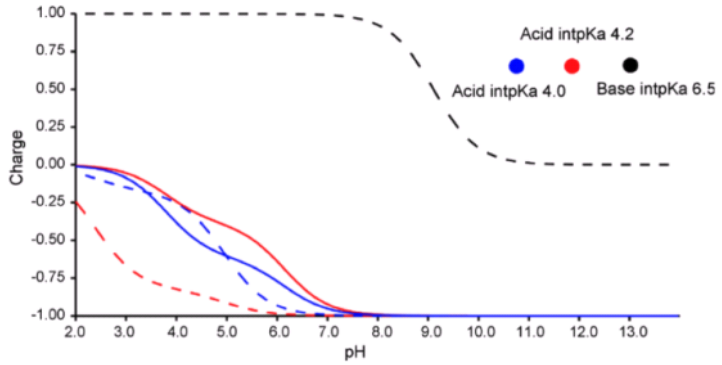
155. (1.50 pts)
The Michaelis-Menten constant, K_m, is independent of the identity of the enzyme. Thus, K_m values can be used to compare any 2 enzymes that operate on the same substrate.

- ( ) True
- (●) False

156. (1.50 pts)
Increases in temperature would be expected to alter the maximum enzyme rate since higher temperatures lower the activation energy barrier required for enzyme binding

- ( ) True
- (●) False

157. (4.00 pts)
In the unfolded state of a protein, all titratable amino acid side chains are exposed. In the folded state, these amino acids could be buried deep within the protein and have no exposure to the aqueous solvent. You find such a coupled system consisting of three residues in a straight line (blue, red, black). The following graph represents a computational model of a titration of this coupled system. Select all of the following choices that are TRUE.



The intrinsic pKa is represented by intpKa. Residues are referred to (blue,red,black) as indicated in the figure.
Credits Nielsen et. al.

(Mark **ALL** correct answers)

- [ ] A) The solid lines could represent the titration of individuals residues for red and blue in an unfolded protein since each has a distinct flattening point that corresponds to a pKa.
- [x] B) The dashed lines could illustrate the behavior of the system in a folded protein where the three residues are in a straight line (blue, red, black) in that order.
- [x] C) Removal of the base would result in a "back-titration" effect on the blue and red residue and increase negativity at certain pH values.
- [x] D) A system with the ordering (blue, black, red) would behave similarly to a system with the ordering (red, black, blue) when titrated.
- [x] E) A system with just red and black in a hydrophobic pocket of a protein would likely have shifted pKa's such that the pKa of red was lower and the pKa of black was higher.

CRISPR Multiple Choice

158. (0.50 pts) Cas9 is most commonly associated with which CRISPR-Cas system?

- ○ A) Type I
- ◉ B) Type II
- ○ C) Type III
- ○ D) Type IV

159. (0.50 pts) Archaea typically use which CRISPR-Cas system?

- ○ A) Type I
- ○ B) Type II
- ◉ C) Type III
- ○ D) Type IV

160. (1.00 pts) Which of the following would serve the most similar function to a Cas9 endonuclease?

- ○ A) DNA polymerase -- 5' to 3' exonuclease activity
- ○ B) RNA polymerase -- 5' to 3' endonuclease activity
- ○ C) Restriction endonuclease -- recognizes a unique 6 bp site and generates staggered DSB cuts
- ◉ D) Restriction endonuclease -- recognizes a unique 6 bp site and generates blunt end DSB cuts

161. (1.00 pts) Which of the following would serve the most similar function to a Cas12a / Cpf1 endonuclease?

- A) DNA polymerase -- 5' to 3' exonuclease activity
- B) RNA polymerase -- 5' to 3' endonuclease activity
- C) Restriction endonuclease -- recognizes a unique 6 bp site and generates staggered DSB cuts
- D) Restriction endonuclease -- recognizes a unique 6 bp site and generates blunt end DSB cuts

162. (1.00 pts)
The protein dCas9 can be converted into a nickase through a _____ mutation. This will allow the protein to make _____ stranded DNA cuts. This prevents indel formation since _____.

- A) E10A; double; staggered DSBs leave sticky ends and facilitate quick repair
- B) H840P; single; there is no NHEJ
- C) A10E; double; staggered DSBs leave sticky ends and facilitate quick repair
- D) A840H; double; blunt end DSBs are quickly rejoined by NHEJ
- E) A840H; single; there is no NHEJ

A researcher fuses a transcriptional inhibitor of the *dubs* gene, LOSS, to dCas9 using the XTEN linker. To examine expression levels, the researcher creates three different conditions:

(1) dCas9-LOSS

(2) dCas9-GFP

(3) dCas9

She expresses each in separate cell lines with LOSS knocked out-- assume complete transfection and no issues in expression levels. Answer the following questions.

163. (0.50 pts) Under fluorescence microscopy, which cell line and organelle would be visible?

- A) Cell line 1: nucleus
- B) Cell line 2: nucleus
- C) Cell line 3: nucleus
- D) Cell line 2: mitochondria
- E) Cell line 1: ribosomes

164. (2.00 pts)
The researcher designs an sgRNA sequence that targets upstream of the *dubs* gene. Assume that an appropriate PAM sequence exists in this region. She then performs RT-qPCR (real time quantitative PCR) to measure expression levels of the *dubs* gene. Rank each condition from least to greatest time required to reach the critical threshold, C_t.

- A) 1 > 2 > 3
- B) 2 > 3 > 1
- C) 3 = 2 = 1
- D) 3 = 2 > 1
- E) 1 > 3 = 2
- F) 1 = 3 > 2

165. (2.00 pts)
The researcher then designs an sgRNA sequence that targets downstream of the *dubs* gene. Select the choice that most accurately models the results of the RT-qPCR experiment from the question above.

- A) 1 > 2 > 3

B) 2 > 3 > 1

C) 3 = 2 = 1

D) 3 = 2 > 1

E) 1 > 3 = 2

F) 1 = 3 > 2

You -- an expert researcher toiling day and night -- manage to discover a 50th CRISPR type (the hypothetical CRISPR type L) in the bacterium *M. ayank* that you know differs from all the other types in only **2** stages of CRISPR mediated immunity. In each question, identify the conclusions that are consistent with the experimental observations.

Question 1: You generate a mutant with loss-of-function mutations in Cas1, but find that the bacterium is still able to target exogenous viral sequences. Select ALL statements that are consistent or TRUE.

166. (0.75 pts) Type L CRISPR systems acquire spacers similarly to type II systems relying on Cas9.

True False

167. (0.75 pts) A similar mutation in a type I CRISPR system would likely interfere with spacer acquisition

True False

168. (0.75 pts) Type L CRISPR systems might not rely on the three stage spacer acquisition system typical of most CRISPR systems

True False

169. (0.75 pts) A mutant with a loss-of-function mutation in Cas2 would likely also still be able to to target exogenous viral sequences.

True False

170. (0.75 pts) A mutant with double loss-of-function mutations in Cas1/Cas2 would likely also still be able to target exogenous viral sequences.

True False

Question 2: You identify the likely effector protein in the system and call it Cas100. Further experimentation reveals that Cas100 contains a PAM binding region that typically binds a T-rich PAM. Surprisingly, find that in *M. ayank*, Cas100 is able to bind to DNA and cut in locations that lack the corresponding PAM, but not when expressed in another bacterium. In each question, identify the conclusions that are consistent with the experimental observations.

171. (0.75 pts) Binding to the PAM sequence in Cas100 triggers a conformational change in its lobes similar to Cas9 that allows the activation of the nuclease domains.

True False

172. (0.75 pts) Cas100 could contain a domain that allows for binding to DNA in the absence of base specificity

True False

173. (0.75 pts) *M. ayank* could express a "docking" protein for Cas100 that facilitates DNA binding in regions without a PAM sequence upstream of the homology site

- (●) True
- ( ) False

174. (0.75 pts) Similar observations are noted for dCas9, which can bind DNA that does not contain the PAM sequence, while Cas9 alone cannot.

- ( ) True
- (●) False

175. (0.75 pts) A T-rich PAM is useful for targeting promoter regions in eukaryotic and prokaryotic genomes.

- (●) True
- ( ) False

176. (2.00 pts)
Based on the above observations in the question statements, which two broad stages of CRISPR immunity does the hypothetical type L system differ from types I, II, and III? Select 2 choices.

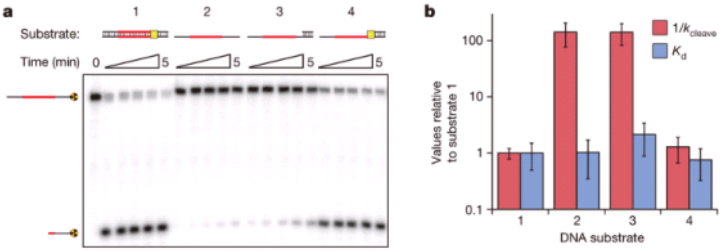
(Mark **ALL** correct answers)

- [x] A) Acquisition
- [ ] B) crRNA processing
- [x] C) Interference

177. (2.00 pts) Provide a justification for your answers to the question above.

Expected Answer: First q -- shows difference in acquisition system since does not use Cas1 or Cas2 Second q -- shows difference in interference since does not require a PAM for high efficiency targeting

You are interested in understanding more about how Cas9 functions as an endonuclease when coupled with guide RNA. You add various DNA substrates *in vitro* along with Cas9-gRNA with a sequence that has homology to all DNA substrates. Red regions in figure (a) denote complementarity to the gRNA, while the yellow box denotes the presence of a PAM sequence for Cas9.



178. (1.50 pts) Which substrates are degraded with high affinity? Select all that apply.

(Mark **ALL** correct answers)

- [x] A) 1
- [ ] B) 2
- [ ] C) 3
- [x] D) 4

Which of the following is consistent with the experimental observations? Indicate if each of the following statements is TRUE or FALSE based on the data provided.

179. (0.75 pts) The presence of a single stranded substrate with an appropriate PAM sequence does not significantly affect the rate of cleavage.

(●) True ( ) False

180. (0.75 pts) Cas9-gRNA requires binding to a PAM sequence to facilitate nuclease activity

(●) True ( ) False

181. (0.75 pts) The absence of a PAM sequence will result in a large increase in the equilibrium disassociation constant in Cas9 binding to the DNA substrate

( ) True (●) False

182. (0.75 pts) The cleavage assay results are consistent with the hypothesis that PAM recognition facilitates local unwinding

(●) True ( ) False

183. (1.00 pts) The addition of an anti-CRISPR protein (competitive PAM mimic) would result in a higher apparent disassociation constant for all results in figure (b).

(●) True ( ) False

An alternative experiment is proposed to test the effect of partial complementarity on Cas9 binding and a cleavage assay conducted. The 4 DNA substrates all contain PAM sequences, but contain less complementarity to the gRNA:

[1]: 100% complementarity
[2]: 50% complementarity
[3]: 10% complementarity
[4]: PAM only

Identify if the following statements are consistent [TRUE] with the results of this experiment.

184. (0.75 pts) The binding energy, delta G, of Cas9 to substrate [1] is more negative than substrate [2]

(●) True ( ) False

185. (0.75 pts) Substrate [1] would have the highest disassociation constant due to the high complementarity

( ) True (●) False

186. (0.75 pts) Substrate [4] would display little to no binding and low cleavage efficiency

(●) True ( ) False

187. (0.75 pts) Substrate [2] and substrate [3] would likely have partial cleavage adducts that show up in the gel due to partial complementarity

○ True ◉ False

188. (1.00 pts) Substrate [1] in this experiment would have very similar results to substrate [4] in the earlier experiment (Figure a)

◉ True ○ False

Congratulations on finishing the test :)