Instructions (shown before students start the test)

You have 50 minutes to finish this exam and there is no computer exploration of protein structure. This exam accounts for 100% of the event score.

Each participant may use one 8.5” x 11” sheet of paper as a note sheet.

Do NOT visit external sites during this exam. Time spent outside the browser will be tracked and flagged for review if above baseline.

If you have any questions, contact me at nparsan@mit.edu

Per Texas Science Olympiad rules, you must have printed notes for this event. If you are communicating with your partner through a voice or video call, please start it before you begin the test itself.

Significant time spent outside of the browser window is grounds for a penalty or disqualification per TSO policies.

Introduction (shown after students start the test)

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1. (1.00 pts) Which of the following isotopes could be used to radioactively label DNA? Select all that apply.

(Mark **ALL** correct answers)

- ☐ A) 14-C
- ☐ B) 32-P
- ☐ C) 12-C
- ☐ D) 32-S

2. (1.00 pts) Select all of the following amino acids with hydrophobic side chains.

(Mark **ALL** correct answers)

- ☐ A) Valine
- ☐ B) Arginine
- ☐ C) Proline
- ☐ D) Citrulline
- ☐ E) Isoleucine

3. (1.00 pts) Which of the following levels of structure would most accurately define the 3D structure of a protein?

- ○ A) Primary
- ○ B) Secondary
- ○ C) Tertiary
- ○ D) Quaternary

4. (1.00 pts) High temperature, pH, or solute concentrations can all do which of the following to a protein?

- ○ A) Combust
- ○ B) Hydrolyze
- ○ C) Fuse
- ○ D) Denature

5. (1.00 pts) Protein folding can occur in the nascent peptide prior to the release of the entire peptide from the ribosome.

○ True ○ False

6. (1.00 pts) A protein with a low isoelectric point (pI) would have a highly negative charge at neutral pH

○ True ○ False

7. (1.00 pts) Proteins with transmembrane regions such as GPCRs will likely have high proportions of hydrophobic amino acids in regions in the membrane.

○ True ○ False

8. (1.00 pts) What does CRISPR stand for?

○ A) Clustered Regularly Interspaced Short Pam-adjacent Repeats
○ B) Clustered Regularly Interspaced Short Palindromic Repeats
○ C) Clustered Regularly Immune Short Protective Repeats
○ D) Clustered Regularly Inserted Short Partial Repeats

9. (1.00 pts) Select all of the following that can be found in Cas9

(Mark **ALL** correct answers)
☐ A) RuvC
☐ B) HNH
☐ C) 5' exonuclease domain
☐ D) 3' exonuclease domain

10. (1.00 pts) What is the PAM sequence recognized by SpyCas9?

○ A) 5' NGG 3'
○ B) 3' NGG 5'
○ C) 5' NCC 3'
○ D) 3' NCC 5'

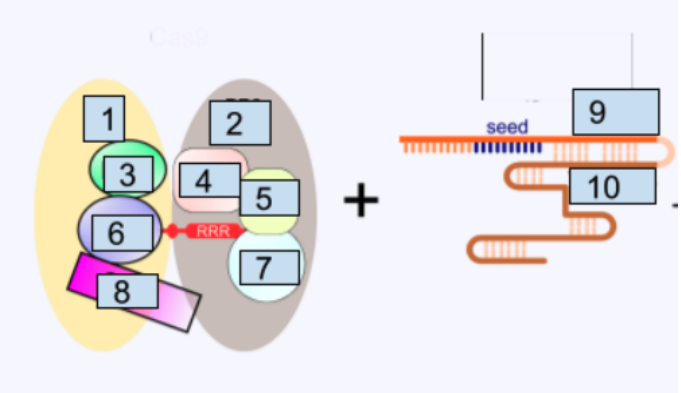
11. (1.00 pts) Which of the following DNA edits would Cas12a (cpf1) perform?

(Mark **ALL** correct answers)
☐ A) Blunt end DSB
☐ B) ssDNA nick
☐ C) Staggered DSB

12. (1.00 pts) Generation of catalytically inactive Cas9, or dCas9, could be performed with two total mutations in which lobes?

○ A) REC1, RuvC
○ B) HNH, REC1
○ C) RuvC, PAM-interacting
○ D) RuvC, HNH
○ E) REC1, CTD

Use the image below to assign structure names to the numerical labels provided.



13. (10.00 pts) Write all answers 1-10 below in order:

1.

2.

3.

4.

5.

6.

7.

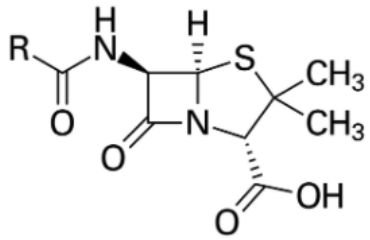
8.

9.

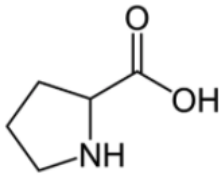
10.

Use the image grid below to answer the following questions.

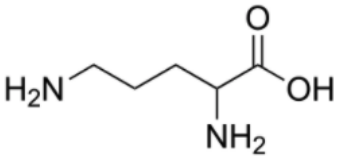
A)



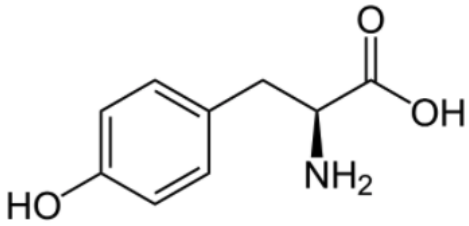
B)



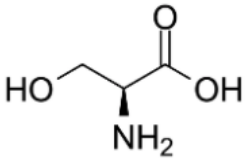
C)



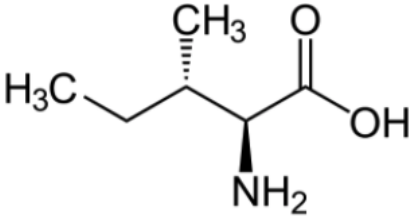
D)



E)



F)



14. (2.00 pts) Select all of the following structures that contain an amide bond.

(Mark **ALL** correct answers)

- ☐ A) A
- ☐ B) B
- ☐ C) C
- ☐ D) D
- ☐ E) E
- ☐ F) F

15. (2.00 pts) Select all of the amino acids have hydrophobic side chains.

(Mark **ALL** correct answers)

- ☐ A) A
- ☐ B) B
- ☐ C) C
- ☐ D) D
- ☐ E) E
- ☐ F) F

16. (2.00 pts) Select all of the amino acids that are frequently phosphorylated.

(Mark **ALL** correct answers)

- ☐ A) A
- ☐ B) B
- ☐ C) C
- ☐ D) D
- ☐ E) E
- ☐ F) F

17. (2.00 pts) Which amino acid has a structural isomer that is indistinguishable using mass spectrometry?

(Mark **ALL** correct answers)

- ☐ A) A
- ☐ B) B
- ☐ C) C
- ☐ D) D
- ☐ E) E
- ☐ F) F

18. (2.00 pts) Which amino acid will absorb radiation of wavelength 280 nm?

(Mark **ALL** correct answers)

- ☐ A) A
- ☐ B) B
- ☐ C) C
- ☐ D) D
- ☐ E) E
- ☐ F) F

19. (2.00 pts) What class of enzymes frequently act on structure E?

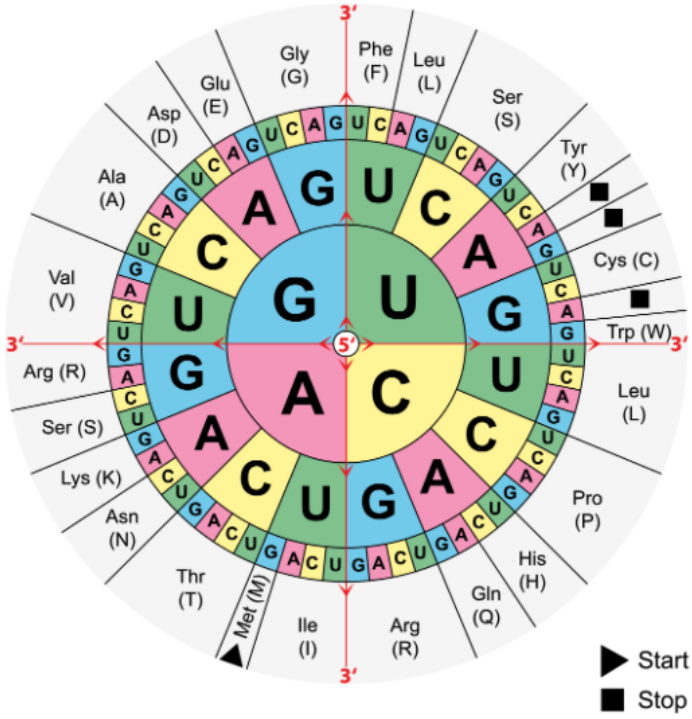
20. (2.00 pts) The carbamylation of the side chain of structure C would have what effect on the pI?

21. (2.00 pts) Provide an explanation for your answer above

You sequence the genome of the rare species M. moopus and discover a highly repeated motif consisting of codons corresponding to hydrophobic and hydrophilic amino acids in a regular pattern. The DNA coding sequence for part of one such motif sequence is provided below:

5' CGC ATG CAG CTG GAA GAT AAA GTG 3'

A codon chart is provided below for reference.



22. (3.00 pts) Provide the protein sequence in ONE letter amino acid code (N → C) that would be generated from the sequence. Do not include spaces.

23. (2.00 pts) At pH 7, what is the net charge on this protein fragment if it were part of a polypeptide backbone?

24. (2.00 pts) At pH 12, what is the net charge on this protein fragment if it were part of a polypeptide backbone?

25. (2.00 pts) At pH 1, what is the net charge on this protein fragment if it were part of a polypeptide backbone?

26. (3.00 pts)
You isolate RNA from a tissue sample of M. moopus and synthesize cDNA. You then decide to amplify fragments of cDNA containing the exact sequence of DNA shown below:

3' GCG TAC GTC GAC CTT CTA TTT CAC 5'

Which of the following primer combinations would be appropriate for this?

- A) 5'GCGTACGTCGACCTTCTATTTCAC 3' ; 5' AAAAAAAAAAAAAAAAAAAA 3'
- B) 5'GCGTACGTCGACCTTCTATTTCAC 3' ; 5' TTTTTTTTTTTTTTTTTTTTTT 3'
- C) 5'CGCATGCAGCTGGAAGATAAAGTG 3' ; 5'AAAAAAAAAAAAAAAAAAAA 3'
- D) 5'CGCATGCAGCTGGAAGATAAAGTG 3' ; 5' TTTTTTTTTTTTTTTTTTTTTT 3'

27. (5.00 pts)
Your professor informs you that your method of amplifying cDNA would likely not result in whole protein sequences following cloning and expression. Provide an explanation for why this is the case.

After performing a correct protocol, you are left with a set of 2 proteins. You note that when both proteins are mixed together in vitro with DNA, there is DNA binding activity. You tag one protein (A) with rhodamine and another protein (B) with fluorescein to perform FRET.

The peak absorbance / emission wavelengths (nm) for the two dyes are provided below:

- Rhodamine: 490 / 530 nm
- Fluorescein: 530 / 603 nm

28. (2.00 pts) If a solution containing tagged protein A only is excited at 490 nm, what would the wavelength of the peak emission be?

29. (2.00 pts) If a solution containing tagged protein B only is excited at 530 nm, what would the wavelength of the peak emission be?

30. (5.00 pts)
A solution containing tagged protein A and B together is excited at 490 nm. Two peaks of emission are observed, one at 530 nm and the other at 603 nm. You suspect this is due to dimerization. Explain why your hypothesis is consistent with the results above.

You prepare a solution containing tagged proteins A and B and bleach the fluorescein dye. Upon bleaching, the fluorophore is inactivated and will no longer emit light following excitation. You add tagged protein B to the solution in 5x excess of the original amount then excite at 490 nm and record the emission spectra.

31. (3.00 pts)
You suspect that proteins in the A-B dimer can freely exchange with subunit proteins in solution. Following sufficient time to reach equilibrium, identify all emission peaks expected in results consistent with rapid exchange kinetics of dimer subunits.

(Mark **ALL** correct answers)
- ☐ A) 490 nm
- ☐ B) 530 nm
- ☐ C) 603 nm

32. (4.00 pts) Provide an explanation for the choices selected above.

33. (3.00 pts)
You suspect that proteins in the A-B dimer cannot freely exchange with subunit proteins in solution. Following sufficient time to reach equilibrium, identify all emission peaks expected in results consistent with very slow exchange kinetics of dimer subunits.

(Mark **ALL** correct answers)
- ☐ A) 490 nm
- ☐ B) 530 nm
- ☐ C) 603 nm

34. (4.00 pts) Provide an explanation for the choices selected above.

35. (2.00 pts)
Solution A-B is incubated with excess DNA fragments containing the appropriate binding sequence and the procedure above is performed. Given that the dimer tightly binds DNA, what emission peaks are expected?

(Mark **ALL** correct answers)
- ☐ A) 490 nm
- ☐ B) 530 nm
- ☐ C) 603 nm

36. (4.00 pts) Provide an explanation for the choices selected above.

The Michaelis-Menten constant

$$K_m$$

is often thought of as analogous to the enzymes dissociation constant

$$K_d$$

and both are frequently referred to as a measure of the enzymes affinity for the substrate.

The equation for an enzyme catalyzed reaction is presented below. E is a free enzyme, ES is the enzyme-substrate complex, S is the substrate, and P is the product. Reaction rates are provided above (forward) and below (reverse).

$$\mathrm{E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P}$$

Differential equations can be used to describe the rates. For example,

$$\frac{dx}{dt} = 0.5$$

would indicate that the quantity x is increasing by 0.5 units at all t. This would correspond to a linear relationship between x and t.

For each of the quantities below, express the following differential equation in terms of the values above. Be sure to include brackets if indicating concentrations.

1. $\frac{d[E]}{dt}$
2. $\frac{d[S]}{dt}$
3. $\frac{d[ES]}{dt}$
4. $\frac{d[P]}{dt}$

37. (4.00 pts) 1.

38. (4.00 pts) 2.

39. (4.00 pts) 3.

40. (4.00 pts) 4.

41. (3.00 pts) Express the dissociation constant

$$K_d$$

in terms of the provided rate constants.

42. (4.00 pts) The total amount of enzyme,

$$[E_0] = [E] + [ES]$$

. Assuming the steady-state equilibrium holds, express the dissociation constant K_d in terms of [E_0], [S], and [ES].

The formula for

$$K_m$$

is provided below for reference.

$$K_{\mathrm{m}} = \frac{(k_{-1} + k_{\mathrm{cat}})}{k_1}$$

43. (4.00 pts) In what situation could the value of

$$K_m$$

be close to

$$K_d$$

?

44. (5.00 pts) If you choose to use

$$K_m$$

instead of

$$K_d$$

to show binding affinity, would this consistently underestimate or overestimate the binding affinity to the substrate? Explain your answer.

The Linweaver-Burke plot assisted early biochemists in linearizing data to determine enzyme parameters such as K_m and V_max. Despite its usefulness, there are several drawbacks to setting a regression line in this manner.

Namely, values on the right of the graph are collected at [1] substrate concentrations and should thus correspond to [2] reaction rates. If we assume experimental error affects reaction rate v and not substrate concentration, then [3] y-values are highly prone to error.

The Eadee-Hofstee plot expresses a different relationship, but maintains the same principle of relating experimental values to a linear plot. The equation is provided as follows:

v = V_max - K_m * v / [S]

Experimental errors in measuring reaction rates would result in deviations parallel to the axis plotting [4].

45. (4.00 pts) Provide responses to blanks 1-4 below:

1.

2.

3.

4.

Research Literacy

In this section, you will be presented with excerpts from a few works studying CRISPR. You will be tested on how well you can apply your background knowledge of CRISPR to understand the information presented.

DNA capture by a CRISPR-Cas9–guided adenine base editor

Note: ABE8e refers to the new adenine base editor proposed in the study.

Lapinaite A, Knott GJ, Palumbo CM, Lin-Shiao E, Richter MF, Zhao KT, Beal PA, Liu DR, Doudna JA

Deamination assays were conducted using either radiolabeled ssDNA or dsDNA substrates and ABE8e in three different states: apo-ABE8e, ABE8e complexed with a targeting sgRNA, or ABE8e complexed with a nontargeting sgRNA. Apo-ABE8e was able to deaminate only ssDNA, whereas ABE8e RNP was able to modify adenines in ssDNA and dsDNA, in contrast to the absence of trans-dsDNA editing by ABE8e engaged in an R-loop complex. Moreover, the dsDNA deamination is sgRNA sequence independent because a nontargeting ABE8e RNP also deaminates dsDNA adenines, suggesting that stable R-loop formation is not required. To test whether Cas9's interaction with the PAM affects the observed dsDNA editing by ABE8e, we performed an in vitro DNA deamination assay with radiolabeled dsDNA devoid of consensus PAMs. To our surprise, ABE8e RNPs containing both the targeting and nontargeting sgRNA deaminated adenine in dsDNA lacking PAMs in the NTS.

46. (3.00 pts)
Deamination of adenine would result in a conversion to _____, which is recognized by DNA replication machinery as _____, resulting in a base pair edit to ____ following ___ round(s) of replication.

47. (5.00 pts) The general structure of ABE8e is an ssDNA deaminase fused to modified Cas9. Provide a hypothesis as to why apo-ABE8e is only able to modify ssDNA.

48. (5.00 pts) The researchers chose to use a nickase Cas9 instead of dCas9. What was the purpose of including partial catalytic activity in ABE8e?

49. (10.00 pts)
You hypothesize that the search mechanism of this particular Cas9 protein involves transient melting of DNA as the protein “scans” for a PAM motif. Is this consistent with the results presented in the excerpt? If so, provide a thorough explanation as to why, referring back to the experimental results presented.

50. (3.00 pts)
Fusion of Cytidine deaminase was used similarly to catalyze the conversion of cytosine bases to uracil. The immediate correction of the mismatch is prevented by Uracil DNA Glycosylase Inhibitor. If a different deaminase targeted 5-methylcytosine, what protein would have to be fused to the base editor for maximum efficacy?

Thanks for taking this test! If you want to leave any feedback, please do so at https://tinyurl.com/utreg21feedback (https://tinyurl.com/utreg21feedback).