Multiple Choice:

1. Which of the following amino acids has a negative charge at pH 7?
   a. Gln
   b. **Glu**
   c. Ser
   d. Cys

2. Which of the following is an example of secondary structure?
   a. Salt Bridge
   b. Disulfide Bond
   c. Hydrophobic Effect
   d. **Alpha Helix**

3. In which of the following types of enzyme inhibition does the inhibitor bind to the enzyme-substrate complex?
   a. Competitive inhibition
   b. Noncompetitive inhibition
   c. **Uncompetitive inhibition**
   d. Mixed inhibition

4. How many amino acids are directly encoded by the universal genetic code?
   a. 9
   b. 22
   c. 21
   d. **20**
5. Which of the following amino acids is not always used in the L stereoisomer?
   a. Glycine
   b. Glutamic Acid
   c. Alanine
   d. Cystine

6. Which of the following amino acids is most important for tertiary structure?
   a. Serine
   b. Arginine
   c. Phenylalanine
   d. Cystine

7. Which of the following amino acids does NOT absorb light in the near-ultraviolet region of the spectrum?
   a. Tyrosine
   b. Tryptophan
   c. Histidine
   d. Phenylalanine

8. Which of the following estimates is closest to the number of genes in the human genome?
   a. 10,000
   b. **20,000**
   c. 30,000
   d. 40,000
9. Which of the following amino acids is most likely to be encountered in the interior of an unknown protein?
   a. Proline
   b. Serine
   c. Lysine
   d. Leucine

10. How many base pairs are in one turn of a DNA helix?
   a. 5
   b. 8
   c. 10
   d. 12

11. Which of the following is a DNA palindrome?
   a. ACCTATAGGT
   b. ACGACGACG
   c. ATTATCGGCG
   d. ATGCTTCGTA

12. Which of the following individuals gave CRISPR its name?
   a. Philippe Harvarth
   b. Francisco Mojica
   c. John van der Oost
   d. Jennifer Doudna

13. Which of the following supplies the DNA found as CRISPR spacers?
   a. Archae
   b. Bacteria
   c. Viruses
   d. C. elegans
14. Which of the following scientists optimized the Cas9 system for use in mammals?
   a. Feng Zhang
   b. John van der Oost
   c. Francisco Mojica
   d. Raj Paul

15. Which of the following is not one of the domains of Cas9?
   a. HNH
   b. RuvC
   c. CTD
   d. PAM

16. Which of the following amino acids in AcrIIA4 does NOT mimic the backbone of DNA?
   a. Asp37
   b. Lys18
   c. Asp69
   d. Glu70

17. In what organism were CRISPR sequences FIRST identified?
   a. E. coli
   b. H. sapiens
   c. S. pyogenes
   d. H. volcanii

18. Which of the following scientists was a pioneer in the understanding of tracrRNA?
   a. Philippe Harvarth
   b. Francisco Mojica
   c. Emmanuelle Charpentier
   d. Feng Zhang
19. Which of the following three base sequences is found after CRISPR spacers in S. pyogenes?
   a. GTT  
   b. NGG  
   c. AAC  
   d. GCG

20. Which of the following RNA types controls where Cas9 cleaves DNA by acting as a template?
   a. tracrRNA  
   b. sgRNA  
   c. mRNA  
   d. snRNA
Short Answer:

1. Consider the following peptide:
Ile - Ala - His - Thr - Tyr - Gly - Pro - Phe - Glu - Ala - Met - Cys - Lys - Trp - Glu - Ala -
Gln - Ala - Asp - Gly - Met - Glu - Cys - Ala - Phe - His - Asp

a. At what position/s might beta turns or bends occur in the peptide? Briefly explain your answer.

A bend would be most likely at amino acids 6-7 (Gly and Pro) (1pt) because the side chains of these amino acids tend to encourage turning. The Glycine has a lot of conformational flexibility due to no steric hinderance from its H side chain (1pt) and the Proline can force the backbone to turn due to the binding of the R group to the N in the backbone to create a kink. (1pt)

b. Where might a pi helix form?

A pi helix would be most likely at position 20 because the Gly there (1pt) would be least disruptive as an "aneurism" in the side of an alpha helix that bulges out from it (1pt)

c. Where might inter-chain disulfide bonds form?

Disulfide bonds would be most likely at positions 12 (1pt) and 23 (1pt) because there are Cystines there, the only amino acid to make disulfide bonds. (1pt)
2. What happens if you replace some of the Asp and Glu amino acids in the loops between beta sheets of AcrIIA4 with Ala? What happens if you replace them with Lys and Arg?

1 pt for identifying that Ala substitution will lower affinity of AcrIIA4 for Cas9
1 pt for identifying that Lys or Arg substitution will also lower affinity of AcrIIA4 for Cas9
1 pt for identifying that affinity loss after Lys or Arg replacement will be greater than affinity loss after Ala replacement
1 pt for identifying that loss in affinity of AcrIIA4 for Cas9 will increase the activity of Cas9 because AcrIIA4 inhibits its activity

3. What is the role of Asn25 in the beta 1-2 loop of AcrIIA4?

1 pt for recognizing that Asn25 blocks Asp10 of Cas9 from accessing DNA
1 pt for recognizing that Asp10 in Cas9 is a catalytic residue important for cleaving the backbone of DNA
1 pt for mentioning that it blocks the RuvC domain from performing its nuclease activity
4. What activity will Cas9 have if its two nuclease domains are inactivated and a pyrimididine deaminase is attached to it?

1 pt for Cas9 will still bind to target DNA sequences without nuclease domain activity
1 pt for Cas9 will NOT cleave DNA once it is bound to it because nuclease domains are disabled
1 pt for pyrimididine deaminase will remove the amine group from a Cytosine base
1 pt for base will be turned into Uracil (which becomes a Thymine when DNA is copied)
1 pt for this new enzyme can be used to change specific bases in the target genome
Tiebreaker: 1 pt extra credit for a specific application in which this new enzyme could be used (such as treating Cystic Fibrosis)