BI/CH421 Biochemistry I
Exam 4 12/12/2016

Last Name (PRINT):

First Name:

Pg	Topic	Pts	Total possible
3	Multiple choice		12
4	Multiple choice		9
5	Multiple choice		15
6	Multiple choice		12
7	Multiple choice and T/F		15
8	footprinting		10
9	Uracil DNA glycosylase and cordycepin		15
10	A vs Z DNA RNA vs DNA		12
11	A strange DNA polymerase and aminoacyl tRNA synthetase		15
12	sugars		12
13	lab		8
total			135 points

Last Name (PRINT):

First Name:

By taking this exam and writing my name above, I agree to abide by the BU academic code of conduct. I understand that any violation of that code is unethical. Unethical conduct includes discussing the exam with anyone before they have taken the exam or sharing the exam with students taking the course in subsequent years.

Instructions: Read the instructions for each section carefully *before* beginning that section.

1) Write your name above and *at least your last name* on ***every*** page.

2) Write all of your answers in the space provided. If you need additional space, you can write on the back of the SAME page. If you do this, you must write "**ON BACK**" so that we know where to look for your answer.

3) Your answers should be brief and legible. A correct answer that cannot be read cannot receive full credit. Additionally, extremely lengthy responses containing both correct and incorrect statements will be graded accordingly.

4) You must write in pen. If you choose to take the exam in pencil, it will not be accepted for a regrade request.

5) Calculators are not permitted.

Number	Log	Ln
2	0.30	0.69
3	0.48	1.10
4	0.60	1.39
5	0.70	1.61
6	0.78	1.79
7	0.85	1.95
8	0.90	2.08
9	0.95	2.20
10	1	2.30

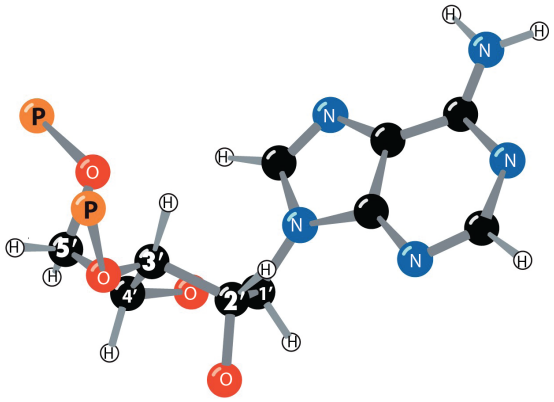
$R = 8.314\ J \cdot K^{-1} \cdot mol^{-1} = 1.987\ cal \cdot K^{-1} \cdot mol^{-1}$

Part I: Multiple choice –Circle the choice that *best* answers the question 3 pts each.

1. Which of the following statements about nucleic acids is *false*?
   a. Nucleic acids are polymers of nucleotides.
   b. Nucleic acids are linked together via phosphodiester bonds to the ribose or deoxyribose sugars.
   c. Nucleic acids are polyanions.
   d. The nucleic acid polymer is asymmetric with two distinct ends, the 5' end and the 3' end.
   e. none of the above (all of the above statements are true)

2. The nucleotide shown below has its ribose in the _____ conformation.

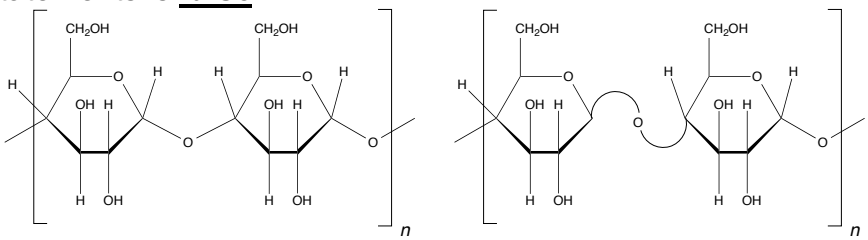
   a. C2'-endo
   b. C2'-exo
   c. C3'-endo
   d. C3'-exo
   e. not enough information



3. For the nucleotide shown above in question 2, the glycosidic bond has the
   a. *syn* conformation
   b. *anti* conformation
   c. major groove conformation
   d. watson conformation
   e. crick conformation

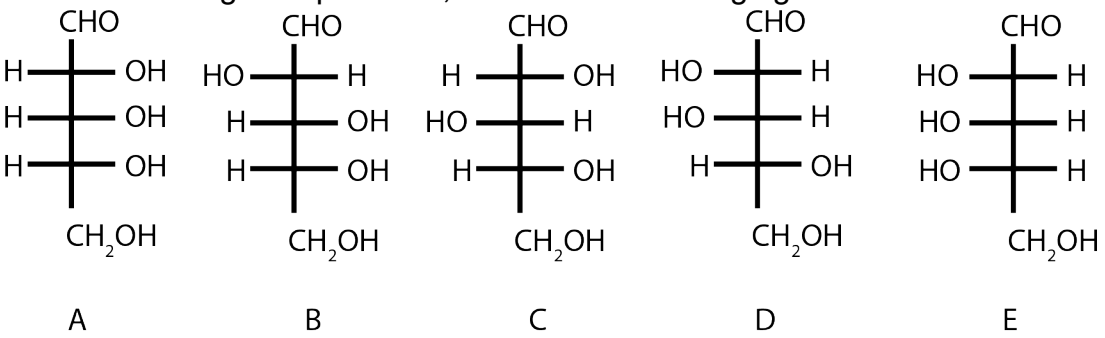
4. The **base** attached to the ribose in question 2 above is
   a. adenine
   b. adenosine
   c. guanine
   d. guanosine
   e. none of the above

5. Using the structures of amylose (left) and cellulose (right), which of the following statements is ***false***



   a. Amylosoe and cellulose both contain *O*-glycosidic linkages.
   b. **The monomeric building block of amylose is a D sugar but the monomeric building block of cellulose is an L sugar**.
   c. Both polymers consist of polymers of glucose.
   d. Both polymers are unbranched polysaccharides.
   e. Amylose has alpha 1→4 linkages and cellulose has beta 1→4 linkages.

For the following two questions, refer to the following figure:

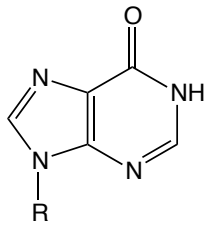


A B C D E

6. D-ribose is
   A) A
   B) B
   C) C
   D) D
   E) E

7. Which of the following statements is *false*?
   A) Sugar A and B are epimers of one another
   B) Sugars A and E are enantiomers
   C) Sugars A and D are diasteromers
   D) **All of the sugars shown are D-aldopentoses**
   E) All of these sugars have three chiral centers

8. If hypoxanthine (shown below) were incorporated into double stranded DNA, it would most likely be found in a watson-crick type base pair with



   a. adenosine
   b. <u>cytosine</u>
   c. guanosine
   d. thymine
   e. inosine

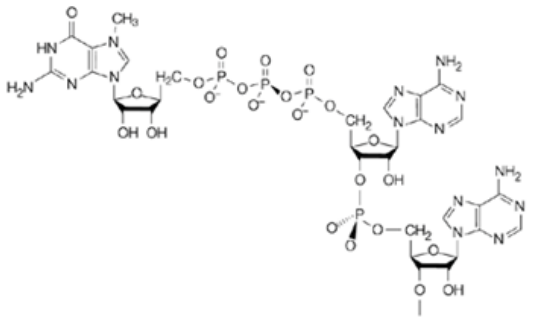
9. Which of the following statements about DNA ligase is *<u>false</u>*?
   a. <u>it forms a phosphodiester bond between a 5' hydroxyl and a 3' phosphate in duplex DNA</u>
   b. It requires a cofactor, either NAD^+ or ATP, depending on the source of the enzyme.
   c. It catalyzes its reaction by a mechanism that involves that involves the activation of a DNA phosphate through the formation of an adenylated intermediate.
   d. It is required for in DNA replication.
   e. None of the above (all of these statements are true)

10. In an A-T Watson-crick base pair, how would the hydrogen bonds change if the adenine base were in its imine tautomer
   a. the keto oxygen would become a hydrogen bond donor
   b. the three hydrogen bonds to guanine would break
   c. the methyl group of adenine would make hydrophobic contact to the thymine
   d. N9 of adenine would become an H-bond donor
   e. <u>none of the above</u>

11. An Okazaki fragment is
   a. <u>a segment of DNA that is an intermediate in the synthesis of the laggings strand during replication</u>
   b. a fragment of RNA that is the subunit of the 30s ribosome
   c. a segment of mRNA that is synthesized by RNA polymerase
   d. a segment of DNA that results from the action of DNA ligase
   e. a segment of DNA that results from exonuclease action

12. You have a double stranded piece of DNA with 15% G. This DNA would be ___% A
   a. 15
   b. <u>35</u>
   c. 42.5
   d. 70
   e. 85

13. Following transcription, eukaryotes cap their mRNA to create the structure shown below:



This cap is comprised of

a. 7-methylguanosine joined to the mRNA via a 5'→3' triphosphate linkage
b. O^6-methylguanosine joined to the mRNA via a 5'→5' triphosphate linkage
c. <u>7-methylguanosine joined to the mRNA via a 5'→5' triphosphate linkage</u>
d. N^6-methyladenosine joined to the mRNA via a 5'→5' triphosphate linkage
e. N^6-methyladenosine joined to the mRNA via a 5'→3' triphosphate linkage

14. Genomic DNA is _____, resulting in the production of _____.

a. <u>transcribed, mRNA</u>
b. translated, tRNA
c. transcribed, proteins
d. translated, mRNA
e. translated, rRNA

15. Heat-denatured DNA exhibits _____ UV absorbance as compared to double stranded DNA. This effect is called the _____

a. <u>increased, hyperchromic effect</u>
b. decreased, hyperchromic effect
c. identical, annealing effect
d. increased, renaturation effect
e. decreased, denaturation effect

16. Which of the following statements about the elongation phase of protein synthesis is false?

a. transpeptidation is catalyzed by the rRNA
b. the incoming aminoacylated tRNA is first bound to the A site
c. transpeptidation requires attack of the alpha amino group of the amino acid in the A site on the ester linkage of the tRNA in the P site
d. translocation requires an elongation factor
e. <u>At least 4 high energy phosphoryl groups (ATP/GTP equivalents) are expended for each peptide bond formed.</u> *<u>i intended this to be the answer as you hydrolyze one GTP with EfTu and one with Ef-G. however if you count ATP→AMP to adenylate amino acid during charging of the tRNA this would then be true.therefore everyone got full credit for this question no matter the anwer</u>*

17. Formation of the ribosomal initiation complex does ***not*** require
    a. fMet•tRNA^{fMet}
    b. several proteins called initiation factors
    c. GTP
    d. mRNA
    e. peptidyl-tRNA

Part II: True False (2 pts each) Circle T or F to indicate if the statement is true or false.

18. **T** F B-DNA has a 10 base pairs per turn of the helix and a rise of 3.4 Å per base pair.

19. T **F** If you exposed negatively supercoiled DNA to an enzyme that cleaves between the 2' and 3' carbons of deoxyribose, you would expect the linking number of the DNA to change.

20. **T** **F** DNA polymerase, RNA polymerase and aminoacyl tRNA synthetases use adenylated intermediates. *When I wrote this, I meant for it to be false (since DNA and RNA polymerases don't adenylate nucleotides to activate them) but you could argue that when dATP or ATP is added to DNA or RNA polymer respectively, this is an adenylated intermediate. Therefore, everyone gets credit no matter the answer for this question*

21. **T** F Oligosaccharides mediate a variety of biological functions that depend on molecular recognition between the protein and the carbohydrate.

Part III: Fill in the blank. For the following sentences, fill in the blank with the word(s) that best complete the sentence. (2 pt each blank)

22. Hypoxanthine (question 8) is formed by deamination of ____*adenine or adenosine*_____.

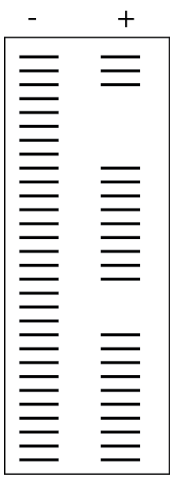
23. During transcription, the template is read in the __3'_____ to ____5'_____ direction.

Part V: Short answer

24. The DNA molecule shown below is a 30mer of double stranded DNA.

**5'-GGATTCTAAT AAAGTAACGT TACGACTTGA-3'
3'-CCTAAGATTA TTTCATTGCA ATGCTGAACT-5'

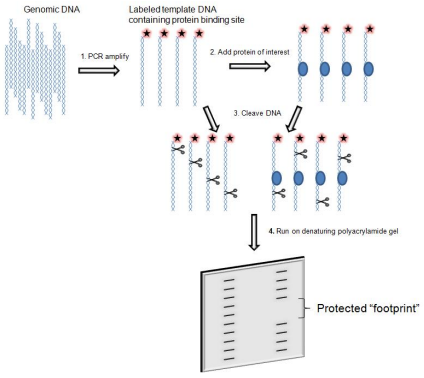
It is believed Protein X binds somewhere in this 30 nt piece of DNA. The duplex DNA is labeled with radioactive 32P at its 5'-end of the top strand(**), then subjected to a footprinting experiment. In the gel, you can only detect bands having the radioactive label.



a) In the gel shown, the direction of migration of the bands is from the top toward the bottom. Based on this information, you can say that the bands at the top of the gel are longer than the bands at the bottom of the gel. (circle an answer) 2 points

True False not enough information given

b) In the gel shown, the 30mer DNA duplex is treated with DNaseI, which can cleave the phosphodiester bonds of the DNA. This cleavage of the DNA strands is completed in the presence (+) and absence (-) of protein X. Based on the pattern of bands shown, explain why some bands are missing when the footprinting experiment is completed in the presence of the protein. (4 points)



When the protein binds to the DNA, it protects the DNA from being cleaved by DNase. Therefore, the protein leaves a "footprint" where it was bound and since the bases it is bound to cannot be cleaved you do not have fragments derived from cleavage at those positions

c) Based on the gel shown, draw boxes around the regions of the duplex DNA shown below where Protein X binds. (4 points)

5'-GGATTCTAAT **AAAGTAACGT TA**CGACT**TGA-3'
3'-CCTAAGATTA **TTT**CATTGCA AT**GCTGA**ACT-5'

Fragments generated in the presence of protein from this DNA:
1 nt: **5'-G (very bottom of gel)
2 nt: **5'-GG (second from bottom)
3 nt: **5'-GGA (third from bottom)
etc. until
10 nt: **5'-GGATTCTAAT; then **no bands at 11, 12, 13 nucleotides because protected, leaving 3 band gap/footprint**
13 nt: **5'-GGATTCTAAT **AAA**
14 nt: **5'-GGATTCTAAT **AAA**GT …etc

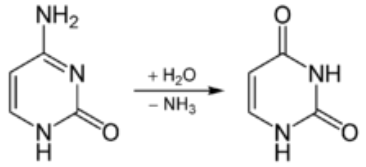
25. Uracil DNA glycosylase is required to prevent C:G to T:A mutations.
a. what is the reaction catalyzed by uracil DNA glycosylase? You can draw structures, write words, whatever you would like to answer the question (5 points)

*hydrolyzes a **glycosidic linkage or N-glycosidic bond***
*U-dexoyribose **in dna** → U + deoxyribose creating **abasic** site*

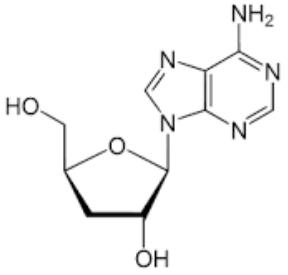
Partial answers like *just* "base excision repair" without details got partial credit

b. explain where the uracil in DNA comes from? feel free to draw some structures or reactions if this helps you to explain. (5 points)

Uracil is created when cytosine spontaneously deaminates or when DNA is exposed to a deaminating agent. See below for structures.

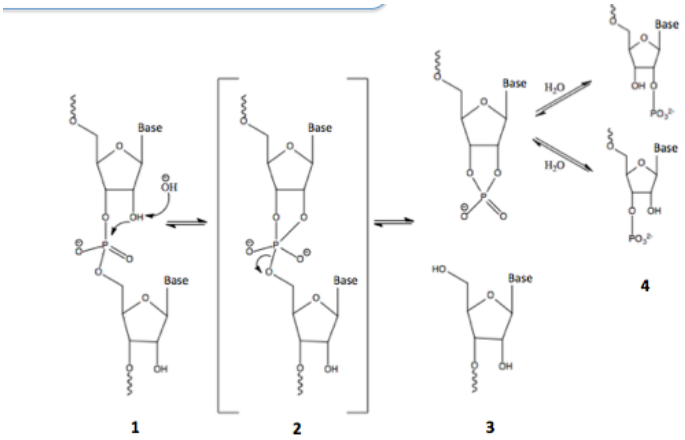


26. Cordycepin, shown below, inhibits bacterial RNA synthesis. Based on its structure, explain its mechanism of action. (5 points)



This looks <u>very similar to adenosine</u> (3'-deoxyribose +adenine). Since it looks like adenosine, <u>it can be phosphorylated to make a triphosphate</u> nucleotide that can get incorporated into RNA. But when the polymerase tries to extend the strand, it can't because <u>it doesn't have a 3'-OH for extension.</u> Must have all 3 underlined concepts for full credit

27. Draw the mechanism by which RNA strands are cleaved by treatment with mild base. Then use this to explain why DNA is more stable in mild base (8 points).



4 points for mechanism (must have structures 1 and 3 but not necessarily 2 or 4).
4 points for idea that DNA doesn't have the 2'-OH and therefore it is less susceptible to base cleavage.

28. Compare and contrast A DNA and Z DNA with respect to the conformation of the glycosidic linkage. (4 points)

In A DNA – all glycosidic linkages are anti (2 points)
In Z DNA – the purines are syn and pyrimidines are anti (2 points)

29. You identify a DNA polymerase that can synthesize DNA in the 3'→5' direction.

a. This new polymerase has proofreading activity imparted by a second active site. Would the proofreading exonuclease activity of this new polymerase be a 3'→5' or a 5'→3' exonuclease? Briefly justify your answer (5 points)

Proofreading for this polymerase would be 5'→3' exonuclease because the new nucleotide would be incorporated at the 5' end of the growing strand so to remove the one just incorporated the polymerase would hydrolyze the 5' nucleotide.

b. Explain why this newly discovered DNA polymerase that could synthesize DNA in the 3'→5' direction would be at a selective disadvantage even if it had proofreading exonuclease activity. (5 points).

If the polymerase grew its chain by adding new nucleotides to the 5' end of the strand then if it makes a mistake and uses the exonuclease activity to hydrolyze its mistakes, the strand would be left with an end with a 5'-P. this cannot immediately react with a new dNTP but first must be activated by pyrophosphorylation (addition of PPi to regenerate 5'-triphosphate) before another round of elongation can occur. Since growth in the 5'→3' direction does not require this activation step after hydrolysis of misincorporated nucleotides, it doesn't need this additional enzymatic activity to polymerize DNA and correct mistakes.

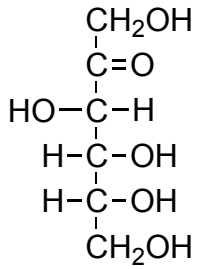
30. Explain why the binding energy of a methyl group (12 kJ/mol) cannot possibly explain the fidelity IleRS which mischarges valine once in every 40,000 turnovers. (5 points).

Valine and isoleucine differ by one methyl group so the $\Delta\Delta G^\circ$ for binding of Ile vs Val is 12 kJ/mol. Due to the relationship between binding energy and K_{eq} (ΔG° = -RT Ln K_{eq}) the ratio of the equilibrium constants between Ile and Val binding is about a factor of 100 (=$e^{\Delta\Delta G/RT}$). Since the enzyme doesn't make a mistake in 1:100 but about 1:10^4 then there must be some additional mechanisms to explain the fidelity (the double sieving mechanism where there are multiple discrimination steps during the aminoacylation reaction and there is an additional proofreading site)

For full credit must indicate that you

1) Understand 12 kJ/mol is the $\Delta\Delta G$ for Ile binding vs Val binding
2) Indicate that they know the relationship between $\Delta\Delta G$ and mischarging. Any of the following relationships will do:
   $K_{eq}^{Ile}/K_{eq}^{Val} = e^{-\Delta G(ile)/RT}/e^{-\Delta G(val)/RT} = e^{-\Delta\Delta G/RT}$
3) Demonstrate that they know how to use this equation (thought don't need to solve it)
4) Demonstrate they understand that this ratio is not large enough (~100) to explain 10^4 turnovers with one mistake.

31. Shown below is the fisher projection of fructose in its linear form.



A) Circle the appropriate description below: (2 points each)

i) Is this D or L fructose? (circle one)

D L none of these

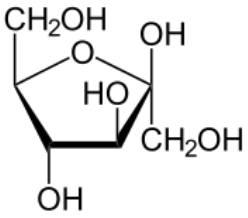
ii) Is fructose an aldose or ketose sugar? (circle one)

aldose **ketose** none of these

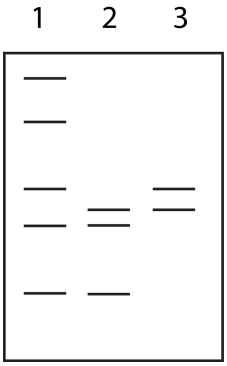
iii) What kind of sugar is fructose? (circle one)

triose tetrose pentose **hexose** heptose

B) Draw Haworth projection of the furanose form of the fructose drawn above as the beta anomer (6 points)

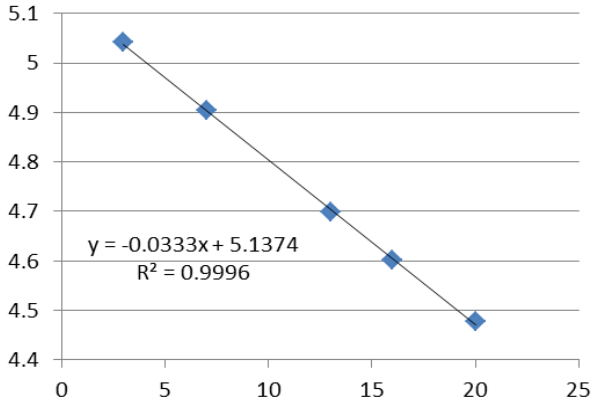


β-D-Fructofuranose



32. You are studying a protein called "protein A". You isolate the protein from cow muscle and analyze it by SDS-PAGE. You analyze a mixture of molecular weight standards (Lane 1, proteins in the sample are 80 kDa, 50 kDa, 30 kDa, 20 kDa, and 10 kDa). You analyze your protein, Protein A, by reducing gel (**with** BME in the sample buffer Lane 2) and nonreducing gel (without BME, Lane 3).

Next, you then analyze the protein via size exclusion chromatography and find it elutes at 7 mL. You also analyze standards of known molecular weight (110 kDa, 80 kDa, 50 kDa, 40 kDa, and 30 kDa; note you have 5 standards, and 5 points on the plot below) and get the following plot for the log of the molecular weight vs elution volume.
Based on the given information, sketch or describe the quaternary structure of protein A. Be sure to include in your sketch or description the molecular weight of the polypeptide chain(s) of protein A and the location of any disulfide bonds. (8 points)



4 pts – from SDS-PAGE you know you have a 25 kDa, 10 kDa and 20 kDa polypeptides (2 pts) and you know the 10 and 20 kDa peptides are in a disulfide bond (2 points)

From size exclusion you know the molecular weight is about 80 kDa. 2 pts for reading MW off graph

The only way you can get 80 kDa is 2x25 kDa + one disulfide bonded 30 kDa = 2 pts however if they got the SEC MW a bit off, as long as the answer written is reasonable (like they could write two 30 kDa disulfide bonded subunits + one 25 kDa for a final MW of 85 kDa) then that is fine. Just want to make sure they know how to use the information given to figure out a quaternary structure.