 NAME:
SID:

Chemistry 135, First Exam
October 23, 2019
Please only turn this page once instructed to do so by the instructor.

This exam will be worth 15% of your overall grade. Please read all of the instructions/questions carefully and answer the question in the space provided or indicated. There should **10** total pages containing **6** multi-part questions. Be sure to transfer any answers you wish to receive credit for to the space provided. No calculators, phones, electronic devices, etc. may be used during this exam. Good luck!

| | **Questions** |
|---|---|
| 1 | 20 points |
| 2 | 52 points |
| 3 | 40 points |
| 4 | 26 points |
| 5 | 16 points |
| 6 | 40 points |
| **Total** | **194 points total** |

Remember that whenever you take an exam, you are really taking *two* tests. The first is a test of your knowledge from the class. The second, and more important, is a test of integrity: that the answers you put down represent your answers and not someone else's. Please make sure to pass the more important test!

You will not need any calculators, phones, electronic devices, headphones, etc. to complete this exam (indeed, they will slow you down), so please make sure these are put away.

The following space is intentionally left blank and may be used as scratch paper, space for a poem, or even an illustration. If you do include work in this area, be sure to transfer any answers you want graded to the provided space in the exam.

**1.** Please carefully evaluate the following statements. If the statement is correct, please mark it as "True." If the statement is false, please provide the correction(s) that renders the statement true. (4 points each)

*For example*:
The chemical biology program at Stanford is the best!
This would be marked as "FALSE" and could be corrected in the following way:

The chemical biology program at ~~Stanford~~ *Cal* is the best!

**a)** __**FALSE**___ RNA polymerase lacks 5' to 3' exonuclease activity. If it had an active site with the ability to perform **3' to 5'** ~~5' to 3'~~ exonuclease chemistry, then RNA polymerase would be able to "proof-read" and correct improperly added bases.

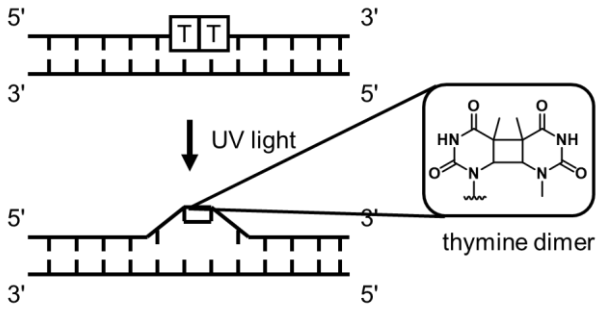
**b)** __**FALSE**___ The primary challenge for introducing unnatural amino acids into peptides is avoiding the proof-reading activities of the **tRNA synthetase** ~~intact ribosome~~.

**c)** __**FALSE**___ The Shine-Dalgarno sequence is a stretch of nucleic acids that is located upstream (on the 5' side) of the **translation** ~~transcription~~ start site.

**d)** ___**TRUE**__ The peptide bond forming step of protein synthesis is catalyzed by the ribosome. Within the active site, it is the ribosomal RNA that catalyzes this reaction, and not the side chains of the protein amino acids.

**e)** ___**FALSE**__ Sigma factors and endonucleases are similar in that they both use interactions between their amino acid side chains and the hydrogen bonding network **on the major and/or minor groove** ~~of the Watson-Crick-Franklin base pair interface~~ to interact with specific regions of duplex DNA.

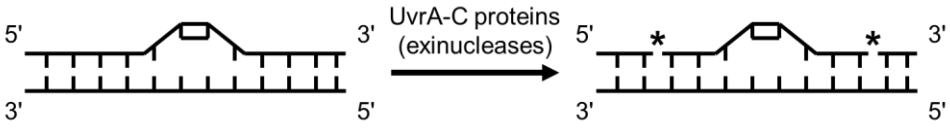
**2.** Thymine dimers form when DNA has been exposed to sunlight. In particular, the ultraviolet wavelengths in sunlight can promote the formation of a cyclobutane ring between two adjacent pairs of thymidines in a stretch of DNA. In general, pyrimidines, rather than purines, are susceptible to formation of dimers.

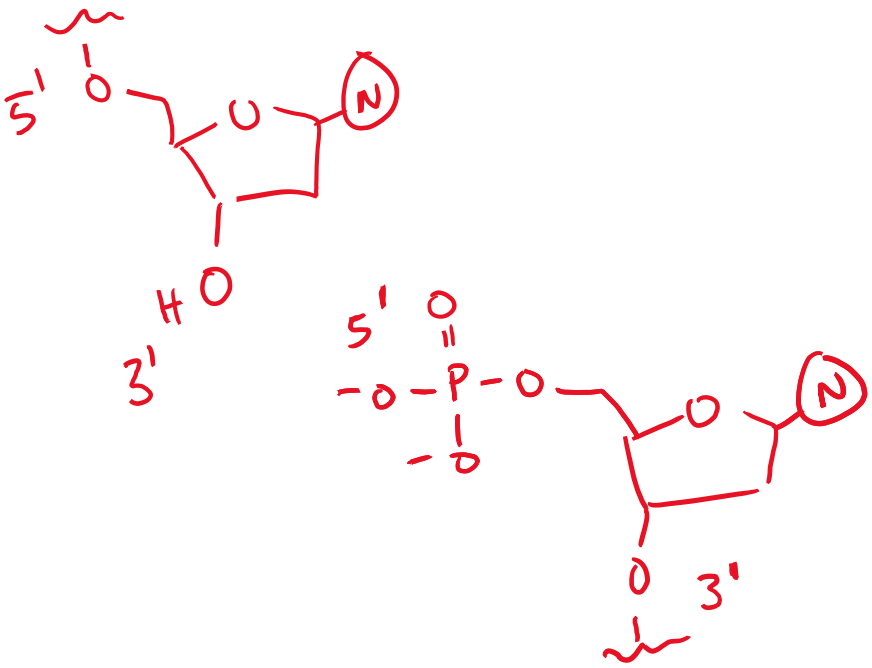



**a)** What other nucleotide in DNA would be likely to form dimers under exposure to UV? Name, structure or abbreviation is okay. (4 points)

**C/cytosine/cytidine**

**b)** In a process that is conserved across species, ranging from bacteria to mammals, nucleotide excision repair mechanisms will repair the thymine dimers. The first step of nucleotide excision repair is the action of the enzyme complex "UvrA-C". UvrA-C is an excinuclease that, like endonucleases we studied in class, catalyzes the cleavage of phosphodiester linkages within a DNA polymer. However, unlike endonucleases, the exinuclease UvrA-C cleaves only one of the strands. In the scheme below, UvrA-C makes two cuts (marked by asterisks, *), on the 5' and 3' side of the thymine dimer. In the space below, draw the ends of the cleaved phosphodiester backbone for one of the cleaved sites, being sure to indicate which is the 5' end and which is the 3' end. You only need to draw the phosphates and ribose; you may abbreviate the nucleobase as "N". (8 points)



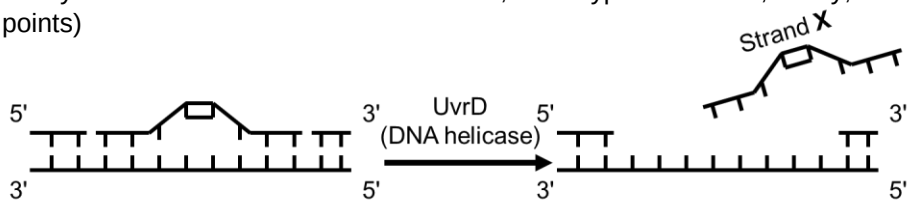
**c)** Based on your knowledge of nucleases, identify the metal ion that would likely be employed by UvrA-C. Provide two specific examples of how this metal ion helps to catalyze the cleavage of the phosphodiester. (1 sentence for each example, 8 points)

**$Mg^{2+}$**

**choose 2 from among the following examples:**

**activates the water nucleophile; activates the P=O electrophile; shields (-) charges on phosphodiester; stabilizes the leaving group; coordinates or aligns the transition state**

**d)** The next step of nucleotide excision repair is catalyzed by UvrD, a DNA helicase. Helicases catalyze the melting of a strand of duplex DNA. UvrD helps to catalyze the dissociation of the strand of DNA that contains the thymine dimer (Strand **X**) from the rest of the genome. Does UvrD help to catalyze the cleavage of any covalent bond? If the answer is no, what types of bonds, if any, does UvrD help to break? (6 points)




**No. It catalyzes the breakage of hydrogen bonds (Watson/Crick/Franklin) that keep the strands together.**

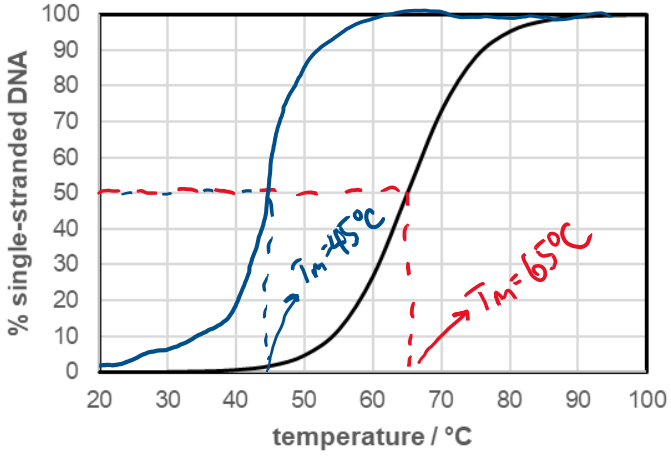
**e)** Figure 1, below, depicts a melting curve for the dissociation of Strand **X** from the complementary region of the genome. Indicate on the graph how you would estimate the melting temperature, or $T_m$, for Strand **X**. What is the approximate $T_m$ for Strand **X**? (4 points)

**Tm is about 65 °C.**

**f)** The melting curve depicted in **Figure 1** is for the uncatalyzed dissociation of Strand **X**. If DNA helicase UvrD is present, what would the melting curve for Strand **X** look like? Draw in this new curve on Figure 1. Show how you would estimate the $T_m$ for the catalyzed reaction. You do not need to worry about the magnitude of the change: just indicate the direction the curve shifts (if you think it moves). (6 points)

**Blue curve**

**Figure 1**



**g)** In the next step of nucleotide excision repair, Enzyme **Y** fills in the missing nucleotides (**Figure 2**). The newly added nucleotides are shown in gray. What is the class of monomers that are used to complete this step? (name or abbreviation is fine) (2 points)

**dNTPs**

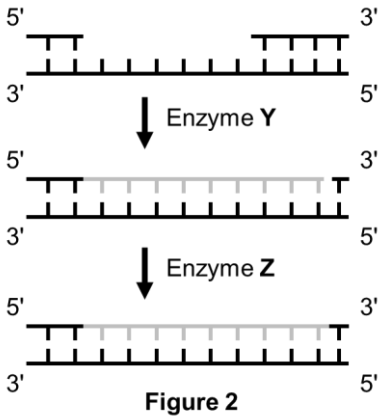


**Figure 2**

**h)** Based on our discussions in class, provide a specific enzyme name for "Enzyme **Y**". (4 points)

**DNA polymerase 1**

**i)** In the final step of nucleotide excision repair, Enzyme **Z** repairs the gap in the phosphodiester backbone that was left behind after Enzyme **Y** completed its work (**Figure 2**). Enzyme **Z** uses ATP to catalyze this step. What is the likely name of Enzyme **Z**? (4 points)

**DNA ligase**

**j)** The last two steps in the nucleotide excision repair process are most like which process we have discussed in class? Select one main Central Dogma pathway and one specific process within this pathway. (6 points).

**Replication; cleanup of lagging strand/Okazaki fragments**

**3.** Nucleic acid bases can form specific H-bond pairs in the context of a DNA duplex (or DNA:RNA duplex).

**a)** What are the specific base pairs that form? You may use single-letter abbreviations. (4 points)

**A and T; C and G**

**b)** These specific base pair arrangements are often referred to as Watson-Crick base pairs, in honor of James Watson and Francis Crick. In Chem 135, we refer to these specific base pair arrangements as Watson-Crick-Franklin base pairs. Franklin, in this context refers to (select one, 4 points):

- **i.** Franklin Delano Roosevelt, who helped fund early parts the seminal study of Watson and Crick through the Lend-Lease Program in the early 1940's.
- **ii.** Franklin Stahl, who, in a remarkable experiment, elucidated the mechanism of DNA replication.
- **iii.** Rosalind Franklin, who first succeeded in crystalizing and collected x-ray diffraction data on B-form DNA.
- **iv.** Benjamin Franklin, who, in addition to inventing bifocals, lightning rods, and the eponymous Franklin stove, made important conjectures about the nature of DNA.

Shown below are two nucleotides incorporated into a strand of nucleic acid. Please do the following:

**c)** Identify whether this nucleic acid polymer is DNA or RNA. (circle one, 2 points)

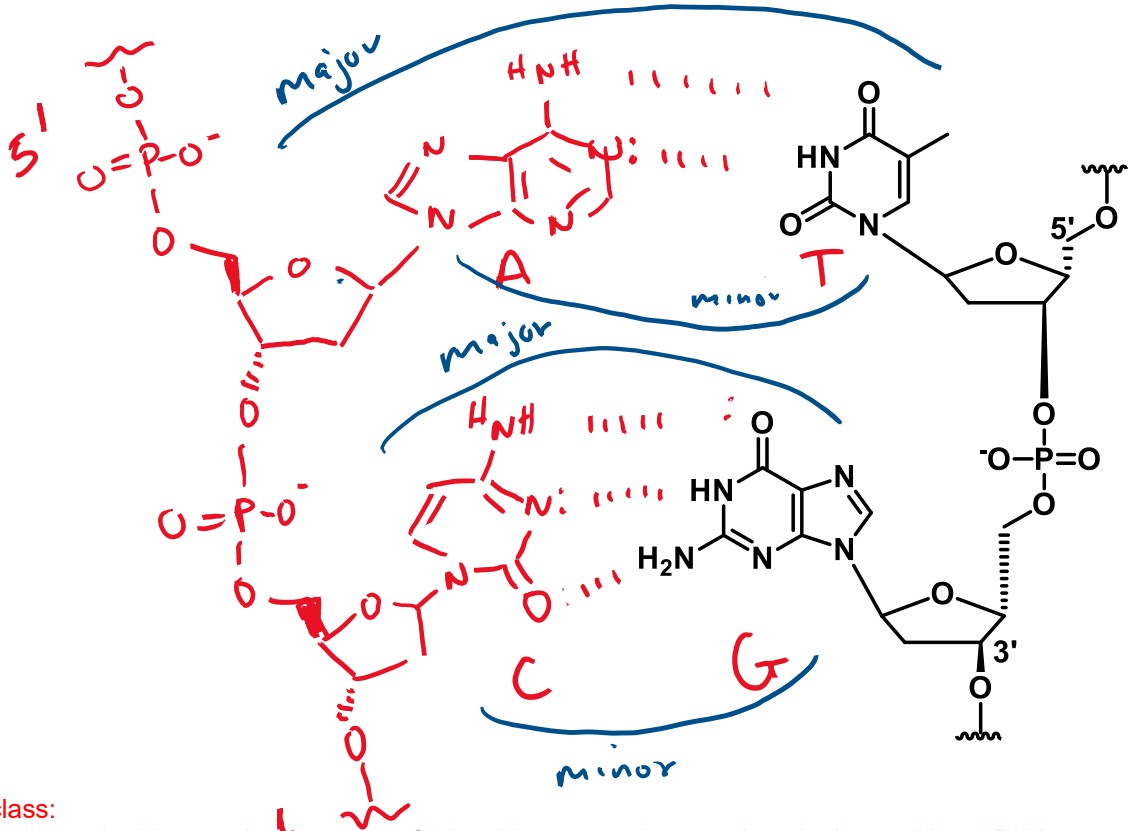
**d)** Draw a complementary strand of DNA, in the orientation that you would expect to find in a duplex, with a careful depiction of the phosphate, sugar, and nucleobases that would be present. (16 points)

**e)** Label the identity of the nucleobases – the ones you drew and the ones already there. Abbreviations are fine. (4 points)

**f)** Indicate the Watson-Crick-Franklin hydrogen bonding patterns that would exist between these base pairs. (4 points)

**g)** Indicate the 5' and 3' ends of the strand of nucleic acid you drew. (2 points)

**h)** For each base pair, indicate the location of the major and minor grooves (4 points)



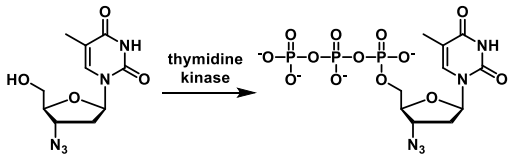

Note to class:
I accidentally made this question far too confusing. I just wanted you to draw in the traditional DNA pairing from our lecture notes. Since my drawing was confusing, we have graded 3d and 3g very liberally. If you got the structure of the nucleobases correct and drew a phosphodiester bond, you should get most of the points.

**4.** Certain viruses, like HIV, are "retroviruses," which means that they encode their genome in RNA. When infecting host cells, they rely on an enzyme called reverse transcriptase, which performs *reverse transcription*, or RNA-templated DNA synthesis. Reverse transcriptase is similar in many ways to DNA polymerase. However, reverse transcriptase has a much higher error rate than DNA polymerase.

**a)** What function in reverse transcriptase is missing that might account for this increase in error rate? What is the name of the type of enzyme (or type of activity) that performs this function? (6 points)

**Proof reading; 3' to 5' exonuclease activity**

**b)** AZT, 3'-azidothymidine, is a drug that can be used to treat HIV. AZT is taken up into cells, where it is phosphorylated by thymidine kinase to generate AZT-triphosphate. AZT-triphosphate is a substrate for reverse transcriptase. What happens to the process of *reverse transcription* when the reverse transcriptase of HIV uses AZT-triphosphate as a substrate? Why does this happen? (1-2 sentences, 8 points)




**Reverse transcription will stop/terminate, if AZT triphosphate is used as a substrate. AZT has no 3' OH, so DNA polymerization cannot continue.**

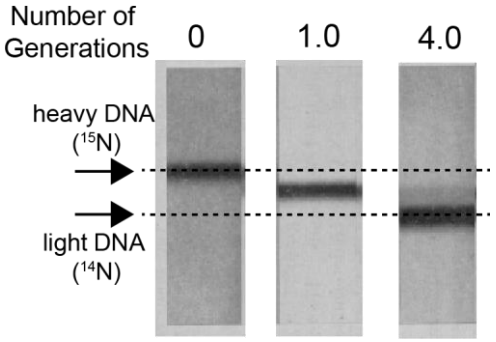
**c)** AZT-triphosphate, and the mode of action you identified above, is *most* similar to which of the technological/chemical biology applications we discussed in class: PCR, Sanger DNA sequencing, DNA origami, or unnatural amino acid incorporation? Briefly explain your answer (1-2 sentences, 6 points).

**Sanger sequencing; both Sanger sequencing and the mode of action of AZT rely on substrates that lack a 3' OH. This prematurely terminates DNA polymerization.**

**d)** At high doses, AZT might affect which other *cellular* enzyme? This would have the most profound consequences for which of the three "Central Dogma" processes we discussed in class? (6 points)

**DNA polymerase; replication.**

**5.** The twisted ladder, or double helical, structure of DNA determined in 1953 hinted at a possible mechanism of replication (recall the last line in Watson and Crick's publication describing the structure of DNA: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.") However, experimental evidence supporting a model of replication was not obtained until 1958, with the series of experiments performed by Meselson and Stahl (in the Norman W. Church Laboratory of Chemical Biology at Caltech).

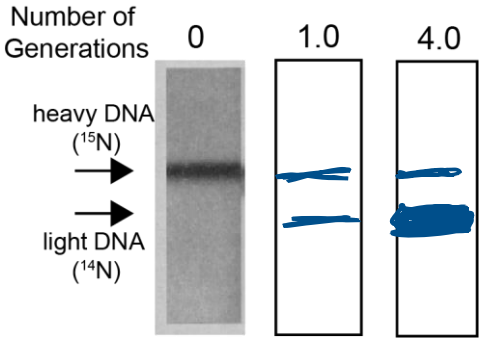



Shown above are the actual data from the Meselson-Stahl experiment we discussed in class (*Proc. Natl. Acad. Sci. USA*, **1958**, *44*, 671-82). In this experiment, bacteria were grown on a food source of heavy nitrogen ($^{15}$N) so that all of their DNA nucleobases were labeled completely with $^{15}$N. Then, the bacteria were switched into normal nitrogen media so that any new DNA would contain light ($^{14}$N) DNA. Meselson and Stahl allowed the bacteria to divide and extracted the DNA at time = 0, 1, and 4 generations, or replications.

**a)** Are the data above consistent with a conservative or semi-conservative model of DNA replication? Briefly explain your reasoning (1-2 sentences). (8 points)

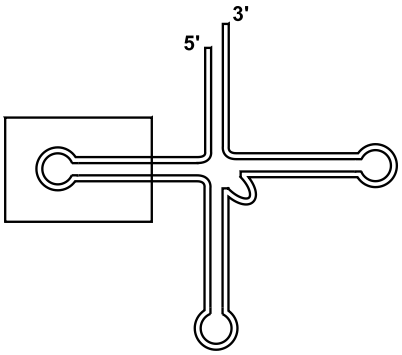
**Semi-conservative, because after 1 generation the new DNA is a mix of light and heavy. With successive generations, the DNA becomes more and more composed of light nitrogen.**

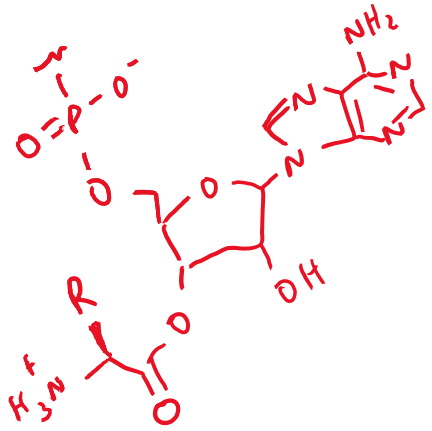
**b)** Suppose that the data Meselson and Stahl had collected looked different. What if it had supported the opposing model? In the space below, sketch out what that data that supports the opposing model might have looked like. (8 points )



**6.** .

**a)** Shown below is a diagram of tRNA. Which end is charged with an amino acid? Provide a detailed structural drawing of the way in which the amino acid is attached. Please draw out the sugar and nucleobase, in addition to the attachment site. You may use "R" as an abbreviation for the amino acid side chain (and you may neglect stereochemistry on the amino acid). (8 points)



**b)** What type of structure is represented in the boxed region, above? What type of nucleic acid sequence is required to form this structure? (4 points)

**Hairpin or stem-loop; palindromic sequences are required.**

**c)** Is the tRNA shown above more likely to be stable in a solution at pH 6.5 or at pH 9? Explain why one of these solutions would make this tRNA much less stable (1-2 sentences, 8 points).

**More stable at pH 6.5. In basic solution, the 2' OH group becomes exceptionally nucleophilic and cleaves the phosphodiester bonds**.

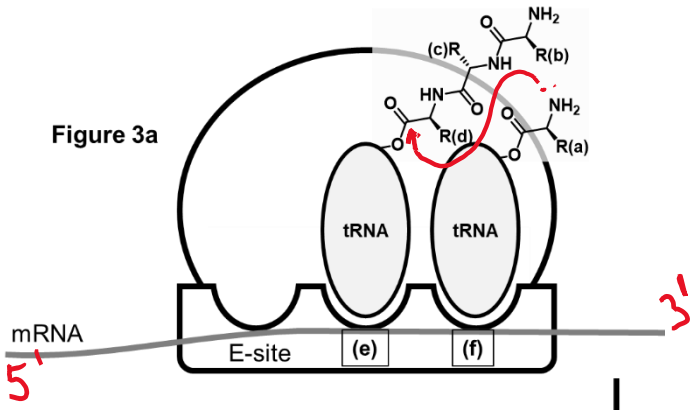
**d)** Which is more likely to cause a change in the primary amino acid sequence, the insertion of a nucleotide into an mRNA transcript, or changing the identity of a single nucleotide in an mRNA transcript? (4 points)

**Changing the identity of a single nucleotide.**

Consider the **Figure 3a** and **3b**, to the right, which depict the process of peptide synthesis.

**e)** Provide a label for the region indicated by **Box (e).** (1 points)

**P-site**




**f)** Provide a label for the region indicated by **Box (f)**. (1 points)

**A-site**

**g)** On **Figure 3a**, label the 5' and 3' ends of the mRNA transcript. (2 points)

**h)** What is the likely identity of the amino acid residue with the side-chain indicated by "**R(b)**"? (4 points)

**Methionine (or fMethionine)**

**i)** The ribosome catalyzes the formation of peptide bonds. In **Figure 3a**, please indicate, using arrow pushing, what acts as the nucleophile and electrophile in this reaction. Then, in **Figure 3b**, draw in the product of this reaction on the ribosome below. (8 points)