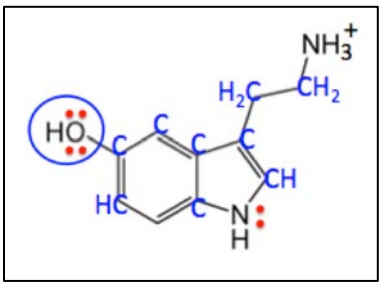
Solution key - 7.016 EXAM 1 (Oct 3, 2018)

Question 1 (8 points)

Serotonin is a neurotransmitter that regulates mood. Low levels of serotonin result in depression and high levels cause anxiety. The following is a partial "line-angle" drawing of serotonin. **Note:** *The hydrogen (H) atoms bonded to carbon (C) and other atoms are not shown but implied.*



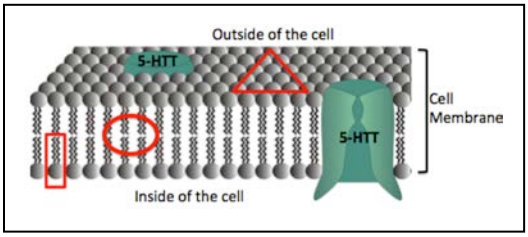
a) Show **ALL** of the C and H atoms on the line angle drawing. (*2pts, 1 for C and 1 for H)*

b) Circle **ALL** groups on the schematic that could participate in condensation reactions. *(2pts: -OH with or without NH_3+ and –NH- OK)*

c) Show the lone pair(s) of electrons on the appropriate electronegative elements taking into consideration the charged states. *(4pts, 2 for each)*

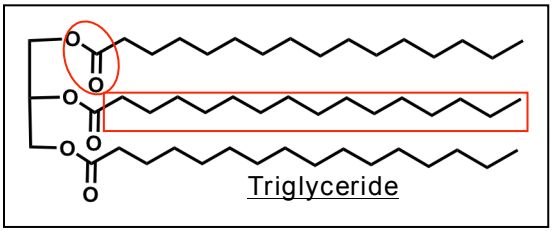
Question 2 (16 points)

The serotonin transporter protein (**5-HTT**) is located in the membrane of serotonin-secreting cells as shown below.



a) Which elements would predominate in the indicated triangular region but **NOT** in circled region of cell membrane and **why**: **C/ H/ *N/ O/ P/* *S***? *(4pts, with 2 for explanation)*
The triangular region represents the hydrophilic, polar head groups, which are compatible with the aqueous exterior of the cell. The elements underlined are part of the hydrophilic groups.

b) The following is the structure of a triglyceride.



i. Circle an ester linkage on the structure. *(1pt)*.
Only one of the three need to be shown

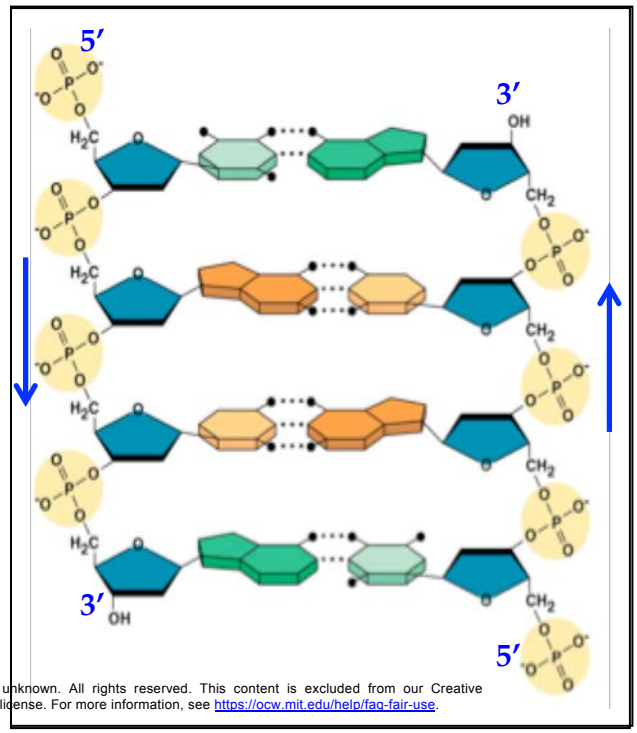
ii. Box one fatty acid chain *(1pt)* in the triglyceride and identify it as **saturated or unsaturated.** *(1pt)*
Only one of the three need to be shown

iii. Your friend says that the triglyceride shown above is a monomer in the boxed area of the cell membrane shown in part (a) above. You are skeptical and rightfully so. **Explain why**.
The cell membrane is a phospholipid bilayer where the polar/ hydrophilic head groups of the amphipathic phospholipid monomers are exposed to the aqueous extracellular region and aqueous cytoplasm of the cell and the hydrophobic fatty acid tails form the inner nonpolar/ hydrophobic inside of the bilayer where they are shielded from the aqueous environment. The triglyceride drawn here is NOT amphipathic i.e. it does not have a polar head groups so cannot for a lipid bilayer. (3pts)

c) Functional 5-HTT includes two polypeptide chains. What is the highest order of the structure of each polypeptide chain of 5-HTT: **Primary/ Secondary/ Tertiary/ Quaternary?** *(2pts)*

d) Secreted serotonin binds to the 5-HTT protein. This causes serotonin reuptake into the cell so that serotonin can no longer act on its target cell. You design a novel competitive inhibitor that covalently binds to 5-HTT. Does increasing the concentration of serotonin reverse the effect of the inhibitor on 5-HTT? **Why or why not?**
No, since the competitive inhibitor and serotonin will compete for binding to the SAME site on the 5-HTT protein. Since the binding is covalent it is irreversible provided you have incubated 5-HTT with the inhibitor before adding the serotonin. (4pts, 2 for each part)

Question 3 (22 points)
The following is a schematic of a part of the SLC6A4 gene that encodes the 5-HTT receptor protein.



a) Label the 5′ and 3′ ends of BOTH DNA strands. *(1pt)*

b) Show the direction of synthesis of **each strand** by drawing arrows. *(1pt)*

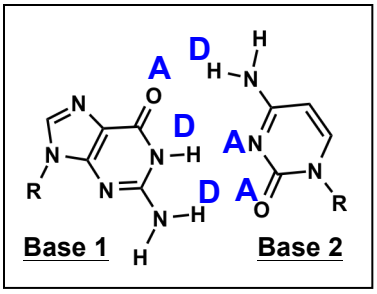
c) Which end of a growing strand of DNA would receive an incoming nucleotide: ***3' or 5'***? *(1pt)*

d) What is the type of interaction between two **adjacent bases** on the **same** DNA strand: **Ionic/ covalent/ hydrogen-bonding/ hydrophobic**? *(2pts)*

e) Chromosomes are made up of DNA and one other class of macromolecule. Which one: **Lipids/ Carbohydrates/ Proteins**? *(2pts)*

f) Explain why it is important for the DNA in a cell to be more stable than RNA and how is this achieved. *Each nucleotide of RNA has a hydroxyl group at the 2'C position, which makes it less stable than DNA. The nucleotides in DNA have an "H" at 2'C position. DNA is the permanent blueprint of our hereditary information, which undergoes one faithful round of replication during cell division cycle. In comparison, multiple copies of the same mRNA transcript are produced from a gene (a DNA sequence) and used to encode protein. Following this, they are degraded i.e. mRNA has a much shorter half-life than DNA. (4pts, 2 for each part)*

g) The following schematic represents a nucleotide base pair in the SLC6A4 gene that encodes the 5-HTT.

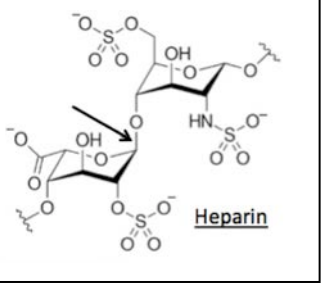


i. Identify the two bases as purine or pyrimidine.

Base 1: ***Purine*** *(1pt)* **Base 2:** ***Pyrimidine*** *(1pt)*

ii. On the schematic, label the hydrogen-bond donors by writing a "**D**" and hydrogen-bond acceptors by writing an **"A"** for each hydrogen bond. *(3pts, 1 for each pair)*

h) The following is the close up view of the molecular structure of heparin.

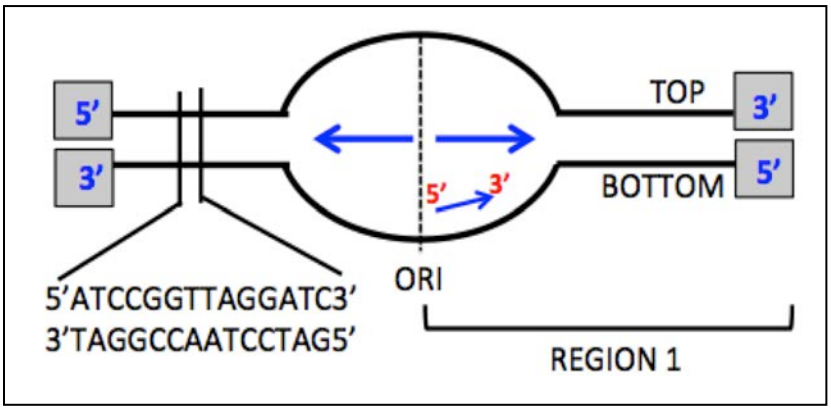


i. Which class of macromolecules is heparin an example of: **Carbohydrates/ Lipids/ Nucleic acids/ proteins.** (*1pt)*

ii. Name the covalent bond that is indicated by the arrow. *Glycosidic bonds (1pt)*

iii. Heparin can be used to purify transcription factors (TFs) that usually bind to DNA. What structural similarity between heparin and DNA makes heparin bind well to a TF? *Both heparin (due to SO_3^- groups) and nucleic acids (due to the PO_4^{3-} of the sugar phosphate backbone) are negatively charged and therefore form ionic bonds with the DNA binding proteins that are rich in amino acids with positively charged side chains.* ***(4pts)***

Question 4 (14 points)
Shown below is a segment of replicating DNA. A small part of the DNA sequence is magnified.



a) Label the **5' and 3' ends** by filling in the shaded boxes and show the **direction of movement of replication forks** by an arrow (→) *(2pts, 1 for each part)*

b) Which strand **in Region 1** is the template for the leading strand: **Top/ Bottom?** *(1pt)*

c) Draw the primer for the leading strand **in Region 1** and label its 5' and 3' ends. *(1pt)*

d) You isolate cells from a normal healthy individual and grow them in the following plates.

- Plate 1 has TAT-2, a compound known to activate telomerase.
- Plate 2 has ciprofloxacin, a compound that inhibits topoisomerase.

In which plate would DNA replication be inhibited and **why**?
Plate 2: In the absence of a functional topoisomerase, the DNA will remain supercoiled and NOT be able to unwind to replicate. In contrast, the cells in Plate 1 will have an active telomerase, which will continue to maintain the chromosomal length. So these cells will not lose any genetic information that may be critical for their growth, response and survival. Hence they will continue to divide and proliferate perhaps leading to the onset of cancer (3 pts with 2 for explanation)

e) You identify a mutant cell that shows an increased error rate during replication.

i. Which enzyme **REMOVES** the incorrect nucleotide during replication and **what activity** of the enzyme allows it to do so?
DNA polymerase (1) due to its 3'→ 5' exonuclease activity (1)

ii. Which enzyme **ADDS** the correct nucleotide to the growing strand of DNA during replication and **what activity** of the enzyme allows it to do so?
DNA polymerase (1) due to its 5'→ 3' polymerization activity (1)

iii. **DNA ligase** is required in all the processes listed below except one.

Circle this process: **Replication/ Proof-reading/ Base excision repair/ Nucleotide excision repair.** *(3 pts)*

Question 5 (22 points)
The following is the DNA sequence of the transcription initiation region of the **SLC6A4 gene**. ***Note:*** ***Promoter sequence*** *is boxed. Transcription* ***begins at and includes*** *the bold and underlined* ***T/A*** *base pair.*

```
5'-TGGACTGCTATAAAAGCAGTACTGCAGAGATGAGGAAAATACGGCCATGGTTCTTAAAGT----3'  TOP
3'-ACCTGACGATATTTTCGTCATGACGTCTCTACTCCTTTTATGCCGGTACCAAGAATTTCA----5'BOTTOM
          Promoter
```

a) Which DNA strand serves as the template strand for transcription and **why**: **Top or Bottom**?
Based on the location of the promoter and the transcription start site, the bottom strand can be read 3'→5' to make an mRNA transcript 5'→ 3' (3 pts with 2 for explanation)

b) Give the first **10 nucleotides** of the **newly transcribed** mRNA transcript and **BOX** the part of the sequence that will be the **start codon** for translation: ***5'UGCAGAGAUG3'*** *(2pts, 1 for each part)*

c) Give the sequence of the anticodon and the amino acid corresponding to the 2nd codon on the mRNA transcript: **Anticodon: 5'*CCU*3'** *(1 pt)* **Amino acid: *ARG*** *(1pt)*

d) Which type of RNA is the anticodon a part of: **messenger RNA/ transfer RNA/ ribosomal RNA?** *(2pts)*

e) You analyze five mutations where the A/T base pair (bold and shaded) at positions **1**, **2**, **3**, **4** or **5**, are marked in the sequence below is substituted by a T/A base pair.

```
                  1      2   3          4    5
5'-TGGACTGCTATAAAAGCAGTACTGCAGAGATGAGGAAAATACGGCCATGGTTCTTAAAGT----3'  TOP
3'-ACCTGACGATATTTTCGTCATGACGTCTCTACTCCTTTTATGCCGGTACCAAGAATTTCA----5'BOTTOM
          Promoter
```

Which of these substitutions will: *(9 pts, 3 for each)*

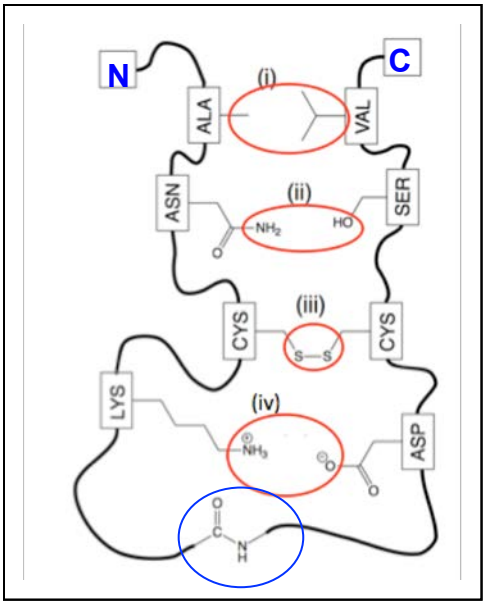
i. Change the sequence of the **SLC6A4 gene** but **NOT** the **SLC6A4 mRNA** transcript **and why**: 1/ 2/ 3/ 4/ 5? *Mutation 1: because it changes the sequence of the promoter, which is a part of the gene but not the mRNA transcript) and Mutation 2: because it is before the transcription start site.*

ii. Change the sequence of the **SLC6A4 mRNA transcript** but **NOT** the 5-HTT **protein and why**: 1/ 2/ 3/ 4/ 5? *Mutation 3: because this is before the open reading frame or outside of the coding region) and Mutation 5: because it is a silent mutation that does not change the actual amino acid sequence in the protein.*

iii. Decrease the responsiveness of 5-HTT to serotonin resulting in depression **and why**: 1/ 2/ 3/ 4/ 5? *Mutation 4: because it creates a nonsense mutation that creates a stop codon and hence produces a truncated 5-HTT. Mutation 1: because it would alter the promoter region which could change the amount of 5-HTT produced thus influencing the protein's responsiveness to serotonin.*

f) You observe that SLC6A4 gene can encode three different proteins in three different cell types. **Explain** in 2-3 sentences how one gene can encode multiple proteins in eukaryotes and **why** this adds to their evolutionary complexity (compared to prokaryotes such as bacteria).
The same gene produces one pre-mRNA that can be alternatively spliced to produce multiple mature mRNA transcripts each of which can be translated into a different protein. This is one of the major mechanisms by which cells can produce a much larger number of proteins relative to the number of genes (20,000 in man). (4pts, 2 for each part)

Question 6 (18 points)
The following are some intra-molecular interactions that are critical for the folding of 5-HTT.



a) Name the **strongest covalent or non-covalent interaction** at the each of the circled positions, (i)–(iv). *(4pts, 1 each)*

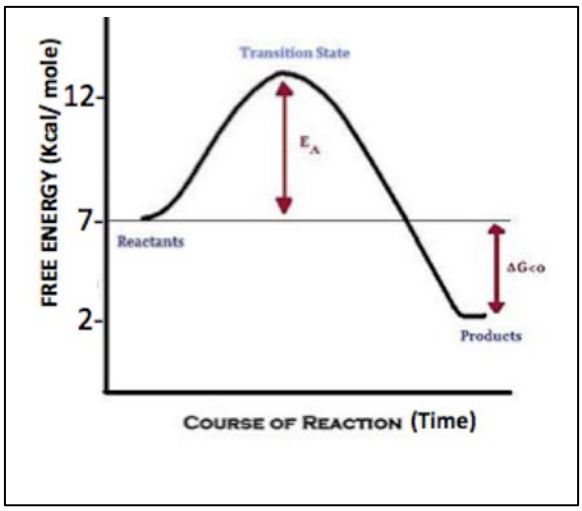
- **Position (i):** ***Hydrophobic interaction***
- **Position (ii):** ***Hydrogen bonding***
- **Position (iii):** ***Covalent bonding***
- **Position (iv):** ***Ionic interaction/ salt bridges***

b) Label the amino (N) and carboxyl (C) ends of 5-HTT in the boxes.
Based on the orientation of the CIRCLED peptide bond, the Ala is nearer to the N terminus and the Val is nearer to the C terminus of 5-HTT (2pts).

c) You treat purified 5-HTT with a protease, which hydrolyzes all of the peptide bonds with a **ΔG = (-) 5 kcal/mole** per peptide bond.

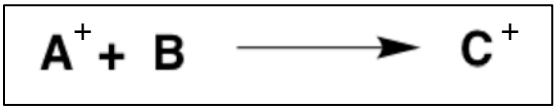
i. Circle all of the correct options. The hydrolysis of peptide bonds is an example of: **Catabolic/ Anabolic / Exergonic / Endergonic** reaction. *(2pts)*

ii. Show the energy diagram for the hydrolysis of a peptide bond and label the: **axes of the plot (including units), ΔG, Energy of activation (E_{act}), substrate energy level (S), and product energy level (P).**



Correct plot: (1pt)
Axes with units: (1pt)
S and P at correct relative free energy: (1pt)
E_{ACT}: (1pt)
Free energy change of (-) 5 kcal/mol: (1pt)

iii. **Explain** how the structure of an enzyme that catalyzes the reaction below might contribute to enzyme catalysis.



In this case the enzyme might lower the energy of activation of the reaction: 1. by binding simultaneously to both substrate molecules to bring them into proximity for the reaction. 2. By stabilizing the high-energy transition state required for the reaction for example by bond distortions. 3. By stabilizing the new charge distribution that occurs as product is being formed. ***(5pts)***

7.016 Introductory Biology
Fall 2018