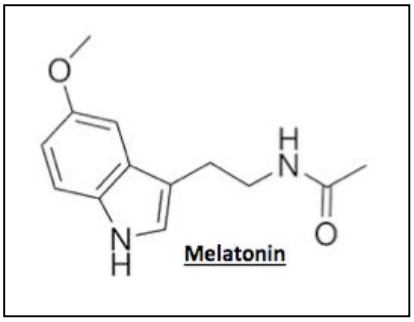
7.013 Problem Set 1- 2018

Question 1

Melatonin is a hormone produced by both plant and animal cells. In humans, it regulates sleep and wakefulness and is used to treat insomnia (inability to fall asleep).

Below is the "line-angle" drawing of melatonin. **Note:** *The carbon (C) and the hydrogen (H) atoms are not shown but are implied.*



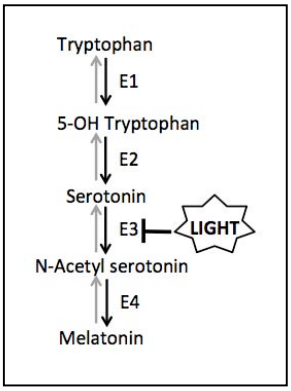
a) Clearly label the position of **ALL** C and H atoms on the line angle drawing and write the **chemical formula** of melatonin in the space below.

b) On the line angle drawing, **box all** nonpolar functional groups and **circle all** electronegative elements.

c) What is the **most likely non-covalent interaction** that allows melatonin to dissolve in aqueous environment?

Question 2

The following diagram shows the biochemical pathway for melatonin synthesis. **Note:** *Enzymes E1-E4 regulate specific steps of the biochemical pathway. You may assume that each reaction step is exergonic ($\Delta G<0$). E3 is inactive in the presence of bright light.*



a) Briefly **explain** why the E1-E4 catalyzed reaction in the absence of light proceeds spontaneously in the forward direction (shown by an **->**) and not in the reverse direction (shown by ->).

b) You identify two patients: **Patient A** who lacks a functional E2 and Patient B who has a hyperactive form of E3 enzyme.

i. Which metabolite would build up in the melatonin synthesizing cells of **Patient A**?

_____

ii. Which metabolite would build up in the melatonin synthesizing cells of **Patient B**?

_____

iii. Which patient would benefit from a melatonin prescription? **Explain** why you selected this patient and not the other.

Question 2 continued

c) Complete the statements below by choosing from the following: **the same/ higher/ lower.** The reaction catalyzed by the hyperactive form of E3 has …

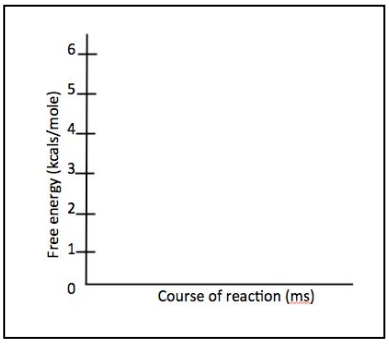
i. _____ **free energy** change as the reaction catalyzed by normal E3*.*

ii. _____ **reaction rate** compared to the reaction catalyzed by normal E3.

iii. _____ **reaction equilibrium** compared to the reaction catalyzed by normal E3

iv. _____ **activation energy** compared to the reaction catalyzed by normal E3

d) For the E3 catalyzed step the free energy change (**∆G) = -2 kcals/mole**.



i. The E3 catalyzed reaction is an example of an **exergonic/ endergonic** reaction.

ii. On the left, draw the energy profile of the reaction catalyzed by E3. Label the reactants (R), products (P), ∆G and activation energy (E_{AC}) of the reaction.

e) E3 is optimally active at pH 7.4 and 37^{O}C. If the same E3-catalyzed reaction was conducted *in vitro* (in a test tube) at pH 7.4 and 50^{O}C, would you expect to see **more/ less/ the same level** of melatonin synthesis? Why or why not? **Note:** *Provide an explanation with respect to the three dimensional (3D-) conformation of E3 enzyme.*

f) You identify two inhibitors of E3: **Drug A** and **Drug B**. Further analysis shows that Drug A alters the 3D-conformation of E3 and prevents it from binding its substrate. Drug A does not bind to the active site of E3.Drug B on the other hand binds to the E3-substrate complex and prevents the formation of products.

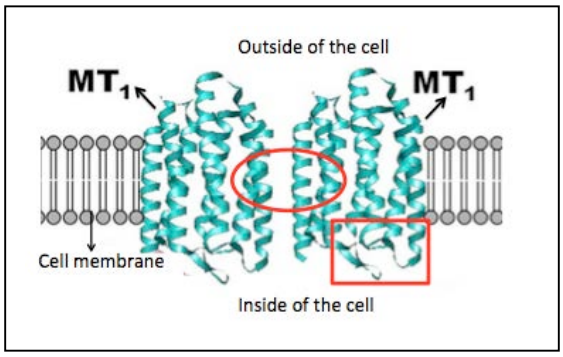
i. Which of the above drugs is an **allosteric inhibitor: Drug A or Drug B**? Why?

ii. Which of the above drugs is an **uncompetitive inhibitor: Drug A or Drug B**? Why?

g) Melatonin is released into the blood stream as it is synthesized. However, it has a short half-life and it is degraded within 20 minutes. **Briefly explain** why it is important for melatonin to have a short half-life in our body. **Note:** *Your answers may vary.*

Question 3

The figure below shows the structure of melatonin receptors (MT_1). The binding of melatonin promotes the dimerization of MT_1. The dimerized form of MT_1 represents its active form.



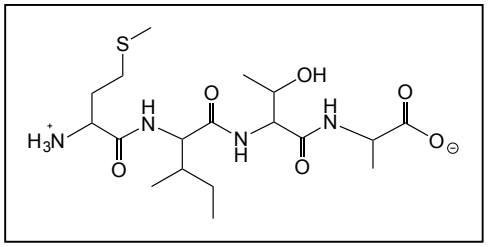
a) What is the highest order of MT-1 structure when it is not bound to melatonin: **primary/ secondary/ tertiary/ quaternary**?

b) What is the highest order of MT-1 structure once it is bound to melatonin: **primary/ secondary/ tertiary/ quaternary**?

c) Does the nature/ characteristics of amino acids inside **the circled region** of MT_1 differ from the nature of amino acids inside **the boxed region**? If so, why?

d) MT_1 has the right 3D- conformation at a pH of 7.4. If MT-1 is denatured at an acidic pH (pH 2.5) in vitro, which level of its structure remains unchanged: **primary/ secondary/ tertiary/ quaternary**? **Explain** why you selected this option.

e) The first four amino acids of MT_1 are shown in the diagram below.



i. On the diagram, show the direction of synthesis of MT_1 by an arrow and box the **first amino acid**.

ii. On the diagram, circle **ALL** the peptide bonds between the amino acids.

iii. Give the **byproduct** of a peptide bond synthesis reaction and classify the reaction as **condensation** or **hydrolysis**.

iv. In the sequence above, name the amino acid(s) that is…

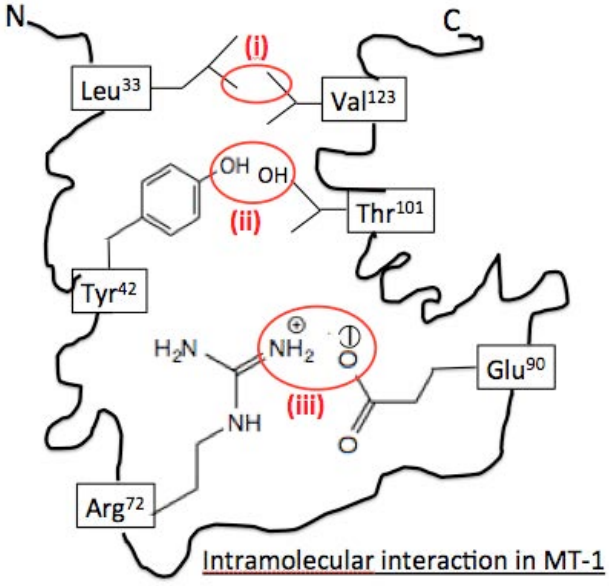
Hydrophilic: _____ **Hydrophobic**: _____

v. **Circle** the group(s) in an amino acid that you considered while answering part (iv) above.

- Amino group ($-NH_2$ group)
- Carboxyl group (-COOH group)
- Side-chain group (R group)
- H atom attached to α-C

Question 4

The diagram below shows the intramolecular interaction between the side-chains of amino acids at positions (i)-(iii) in the MT_1 protein. Each of these interactions is critical for the correct 3D folding of MT_1, which allows it to bind to melatonin.



Intramolecular interaction in MT-1

a) What is the strongest non-covalent interaction at positions (i)–(iii): **Ionic interaction/ hydrophobic interaction/ hydrogen bonding?**

i. _____

ii. _____

iii. _____

b) You identify four individuals (A-D) each of which has either one or two amino acid substitutions at the positions (i) –(iii) compared to the normal MT_1 protein.

Individuals	Amino acid substitutions
A	Leu^{33} -> Ile^{33} & Val^{123} -> Ala^{123} at position (i)
B	Tyr^{42} -> Asn^{42} & Thr^{101} -> Ser^{101} at position (ii)
C	Leu^{33} -> Ile^{33} & Val^{123} -> Trp^{123} at position (i)
D	Glu^{90} -> Lys^{90} at position (iii)

Explain, in terms of the type of mutation whether the mutant version of MT-1 in each of these individuals is likely to fold correctly and bind to melatonin.

c) Based on the mutations in the table above, which individuals (A-D) will have insomnia and **why**?

d) Although the melatonin-binding site of MT_1 is comprised of only a few amino acids, the MT_1 receptor as a whole is comprised of many amino acids. **Explain** how the amino acids outside the melatonin binding site of MT_1 may contribute to the MT_1 function.

Question 5

a) The melatonin receptor exists in different isoforms: MT_1, encoded by *MT-1 gene* and MT_2, encoded by *MT-2 gene.* The following is the sequence corresponding to 90-140 base pairs (bp) of *MT-1* and *MT-2* genes.

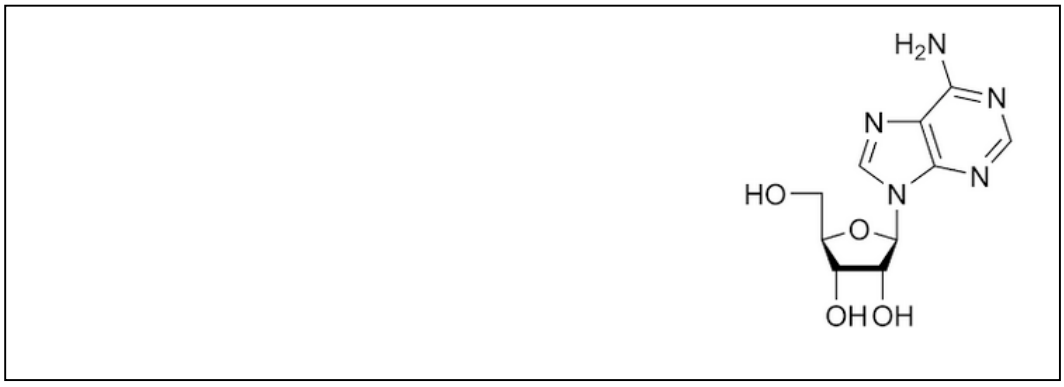
```
                90                                                  140
MT-1: 5'------GATATGCCCCCCCCGGCGCGCGATATGCCCCCCCCGGCGCGCGTGCGTGA-------3'
      3'------CTATACGGGGGGGGCCGCGCGCTATACGGGGGGGGCCGCGCGCACGCACT-------5'

                90                                                  140
MT-2: 5'------GATATGATATATATATATATAGATATGAAAAATTTTTATATAGTGCGTGA-------3'
      3'------CTATACTATATATATATATATCTATACTTTTTAAAAAATTATCACGCACT-------5'
```

i. Which of the above sequence will denature at a lower temperature and **why**?

ii. In the sequences above, which end is the growing end that receives the incoming nucleotide: the **5' <u>or</u>** the **3' end**?

b) If you denature DNA duplex and protein, which macromolecule is likely to renature and **why?**

Shown below is the chemical structure of adenosine, one of the five nucleotides that make up the nucleic acids.



c) On the diagram, label the carbon atoms of the sugar as **C1'-C5'**.

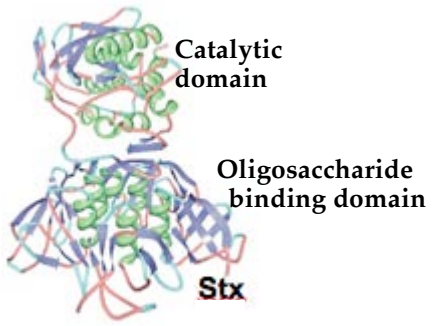
d) On the diagram, add the phosphate groups to 5'C of adenosine to convert it to adenosine triphosphate **(ATP).**

e) Which nucleic acid (DNA or RNA) would the **ATP** in the diagram be a part of and **why**?

f) Circle the group that would participate in the formation of a phosphodiester bond if this nucleotide were **<u>added</u>** to the growing end of a nucleic acid chain.

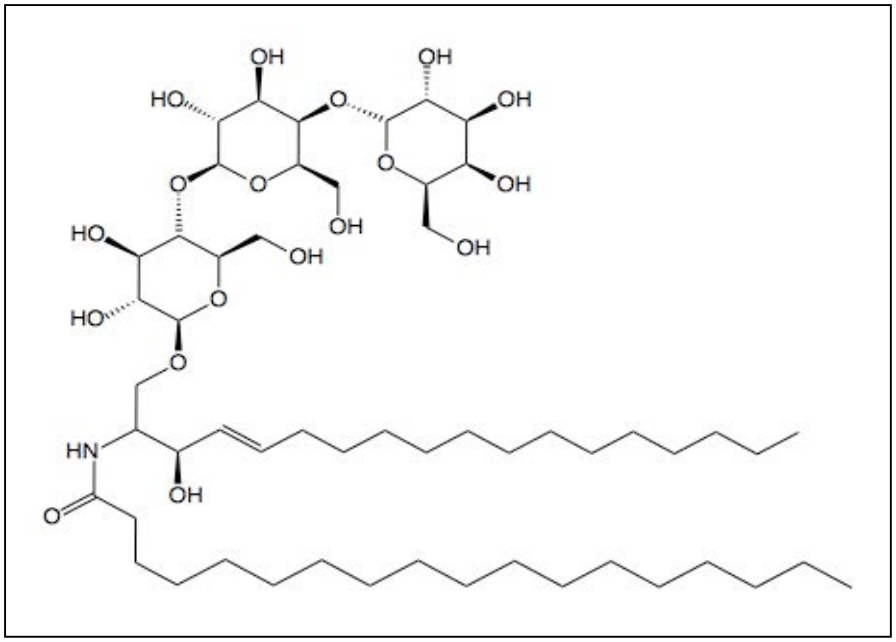
Question 6
Many drugs that promote sleep can cause diarrhea as a side effect.

This side effect can also be mimicked by Shiga toxin (Stx), a protein produced by *Shigella dysenteriae* bacteria. The Stx binds to the cell surface glycolipid Gb3 and inhibits protein synthesis in the target cell. This results in dysentery, which is an infection of the intestine resulting in severe bloody diarrhea.



- The structure of Stx is shown on the left. It has a catalytic domain non-covalently attached to an oligosaccharide-binding domain. (PDB ID: 1DM0. Fraser, M.E., et al. (1994) Crystal structure of the holotoxin from Shigella dysenteriae at 2.5 A resolution. *Nat.Struct.Mol.Biol.* 1: 59-64.)
- The oligosaccharide-binding domain specifically binds to the oligosaccharide part of Gb3 glycolipid and triggers endocytosis of Stx.
- The catalytic domain of Stx is cleaved into two fragments, A1 and A2 by the sequence-specific protease Furin in the target cell.
- The A1 fragment catalyzes the removal of a single purine base (A^{4324}) from ribosomal RNA (rRNA) thereby inactivating the ribosome.

a) The structure of Gb3 is given below. On the schematic…



i. **Circle** a saturated hydrocarbon chain.

ii. Draw an **arrow** to show **one** glycosidic bond.

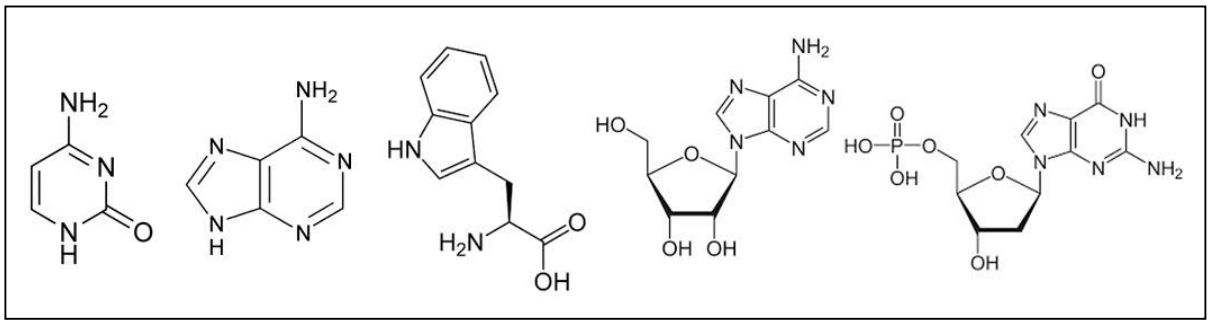
iii. Indicate the hydrophilic end of Gb3 with a **star**.

b) Complete the sentence. The oligosaccharide-binding domain of Stx is mostly composed of ***β-sheets***, which are stabilized by_____ formed between the amide (-NH) group and the carbonyl (-C=O) group of the peptide chain backbone.

c) List **two** amino acid residues you would expect to find at the oligosaccharide-binding site of Stx. **Explain** why you selected these amino acids. **Note:** *Your explanations may vary.*

Question 6 continued

d) The A1 fragment of Stx catalyzes the hydrolysis of the N-glycosidic bond between the ribose sugar and the adenine base of the adenosine nucleotide in RNA. Which of the following is a product of this reaction? **Circle** the correct product(s).



Question 7

The microbiome is defined as the collection of microorganisms that inhabit an environment, creating an ecosystem. The human microbiome is a collection of different bacteria, viruses and many microorganisms. Often, our microbiome defines our health and our response to different drugs.

a) Which of the given options are self-replicating on its own in response to appropriate growth signals: **Bacteria/ viruses/ skin cell**?

b) Identify the organelle within a human cell that generates ready energy in the form of ATP. This organelle is often regarded as a prokaryotic cell that was engulfed by a eukaryotic cell during the course of evolution. List **two features** of this organelle that characterize it as likely to have been derived from a prokaryotic cell

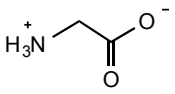
c) In the table below, identify the **organelle(s)** or location(s) within the **skin cell,** where the following proteins function. Please select your options from the following: Mitochondria, Endoplasmic reticulum (ER), Golgi body, Plasma membrane (PM), Cytoskeleton, nucleus, lysosomes.

Proteins	**Organelle(s) / location(s) in which the proteins function**
Proteins that participate in the synthesis of ATP through the process of aerobic (oxygen dependent) respiration	
Proteins that participates in transcription of genes	
Proteins that is involved in digesting toxic material in the cell	
Proteins that adhere identical eukaryotic cells together and participate in intercellular (cell to cell) communication	
Proteins that are a part of a dynamic network of fibers that maintain the shape and motility of a eukaryotic cell	
Enzymes that are involved in modification of newly synthesized proteins	

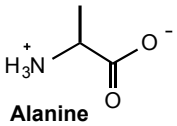
Amino acid table

TWENTY ENCODED
α-AMINO ACIDS
at pH 7

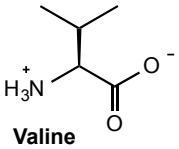
NO side chain



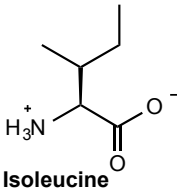
Glycine
Gly (G)



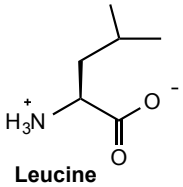
Alanine
Ala (A)



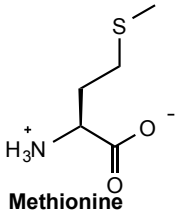
Valine
Val (V)



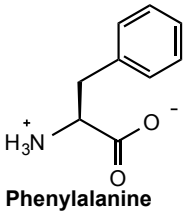
Isoleucine
Ile (I)



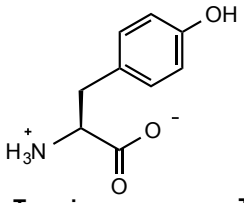
Leucine
Leu (I)



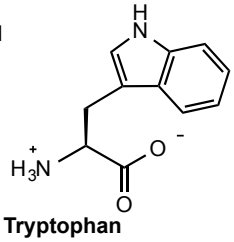
Methionine
Met (M)



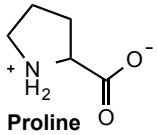
Phenylalanine
Phe (F)



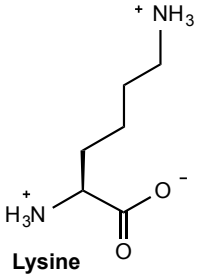
Tyrosine
Tyr (Y)



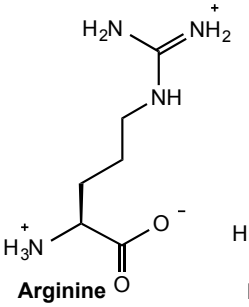
Tryptophan
Trp (W)



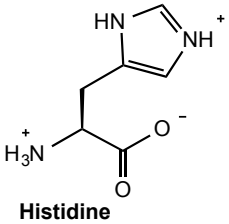
Proline
Pro (P)



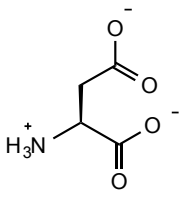
Lysine
Lys (K)



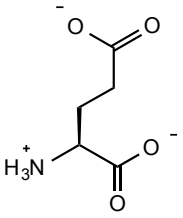
Arginine
Arg (R)



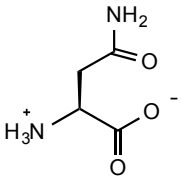
Histidine
His (H)



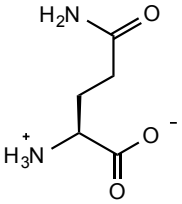
Aspartic acid
Asp (D)



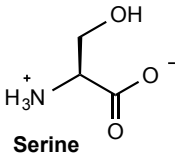
Glutamic acid
Glu (E)



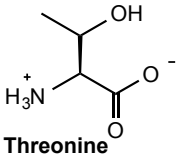
Asparagine
Asn (N)



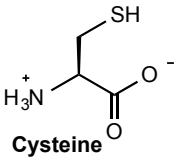
Glutamine
Gln (Q)



Serine
Ser (S)



Threonine
Thr (T)



Cysteine
Cys (C)

7.013 Introductory Biology

Spring 2018