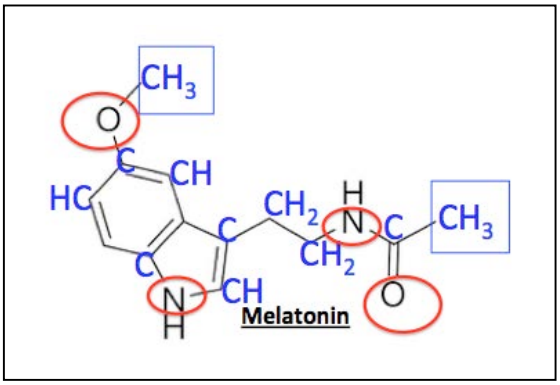
Solution key- 7.013 Problem Set 1- 2018

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.*

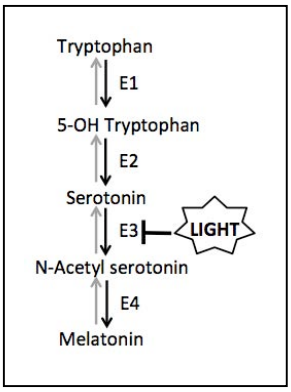


a) Clearly label the position of **ALL** C and H atoms on the line angle drawing and write the **chemical formula** of melatonin in the space below. $C_{13}H_{16}O_2N_2$

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?
Hydrogen bonding

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 ->).
There are multiple correct answers: E1-E4 catalyzed reaction proceed spontaneously in the forward direction since they all involve the hydrolysis of high-energy bonds i.e. they are exergonic ($\Delta G <0$). The reaction in the reverse direction are energy requiring or endergonic $\Delta G >0$ and therefore energetically less favorable. You may also say that in this biochemical pathway the product of one reaction step is the substrate of the next reaction step and is thus being used up. This allows each reaction step to proceed in forward direction to make more products. You may also state that a different enzyme catalyzes the reverse reaction, in the biological system.

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**?
5-OH Tryptophan

ii. Which metabolite would build up in the melatonin synthesizing cells of **Patient B**?
N-Acetyl serotonin (and melatonin)

iii. Which patient would benefit from a melatonin prescription? **Explain** why you selected this patient and not the other.
Patient A is not able to synthesize melatonin since the biochemical pathway of melatonin synthesis, in the absence of functional E2, stops after E1 catalyzed Step 1. This patient may suffer from sleep deprivation and insomnia and hence benefit from melatonin prescription. In comparison, in Patient B, E3 is hyperactive perhaps resulting in normal/ more melatonin synthesis. So this patient will not suffer from insomnia and hence not be benefited from exogenous melatonin.

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 …

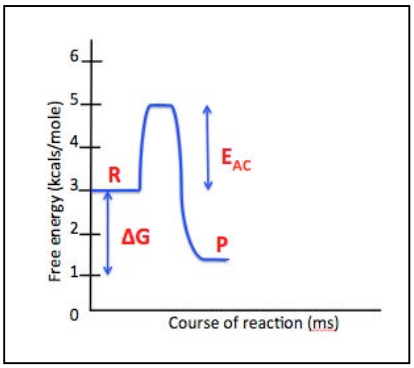
i. ***The same*** **free energy** change as the reaction catalyzed by normal E3**.**

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

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

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

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



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

Note: *This reaction has a negative free energy change, which makes it exergonic.*

ii. On the left, draw the energy profile of the reaction catalyzed by E3. Label the reactants (R), products (P), ΔG and the 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.*

It is possible that a change in the temperature would denature the enzyme. In the absence of the enzyme the activation energy will not be lowered thus reaction will either not proceed or will proceed at a very slow rate. You can also argue that an increase in temperature may increase the random movement of the substrate (ΔG= ΔH- TΔS) thus reducing the free energy. This may favor the reaction progress (but this in a biological system is less likely).

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?

Drug A is likely an allosteric inhibitor since it binds to the site on E3 that is different from the substrate-binding site. As a result the 3D conformation of E3 is altered and it is unable to bind to its substrate.

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

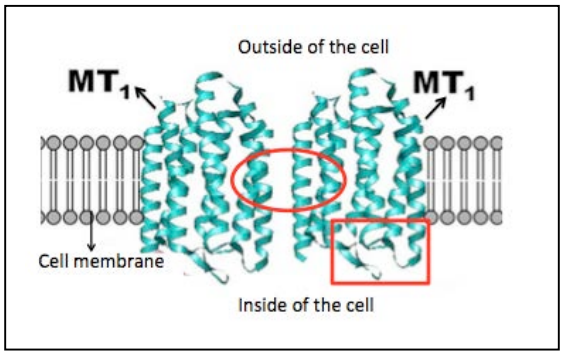
Drug B since it does not bind to the free E3 enzyme but instead to the enzyme-substrate complex to alter its conformation. This prevents the formation of the product.

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.*

Melatonin promotes sleepiness. If it were not degraded then a person would be sleepy all the time. One needs a normal sleep-wakefulness cycle in life!

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**? *Tertiary*

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

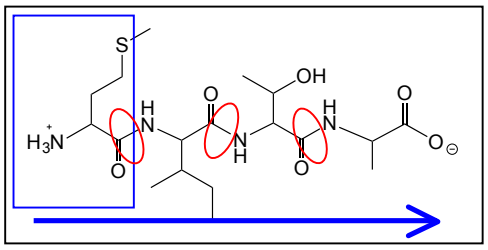
c) Does the nature/ characteristics of amino acids inside **the circled region** of MT_1 differ from the nature of amino acids inside **the boxed region**? If so, why?

MT_1 appears to be a transmembrane protein since it spans the plasma membrane. The amino acids of MT_1 that are within the circled region are in contact with the hydrophobic tails of the phospholipids molecule that make the lipid bilayer/ plasma membrane. So these amino acids are likely to have hydrophobic side-chains that are compatible with the hydrophobic interior of the cell membrane. In comparison, the amino acids of MT_1 that are in the boxed region will likely have hydrophilic side-chains so that they are compatible with the hydrophilic environment of the cytoplasm.

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.

A change in the pH disrupts the 3D- conformation of the protein by disrupting the non-covalent interactions (hydrogen bonding, hydrophobic interactions, ionic interaction, VDW forces) but it usually does not disrupt the peptide bonds, which hold the amino acids together to form the primary structure of the protein. So the primary sequence of amino acids in MT_1 will remain unchanged.

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**. *Water is the byproduct of the condensation reaction.*

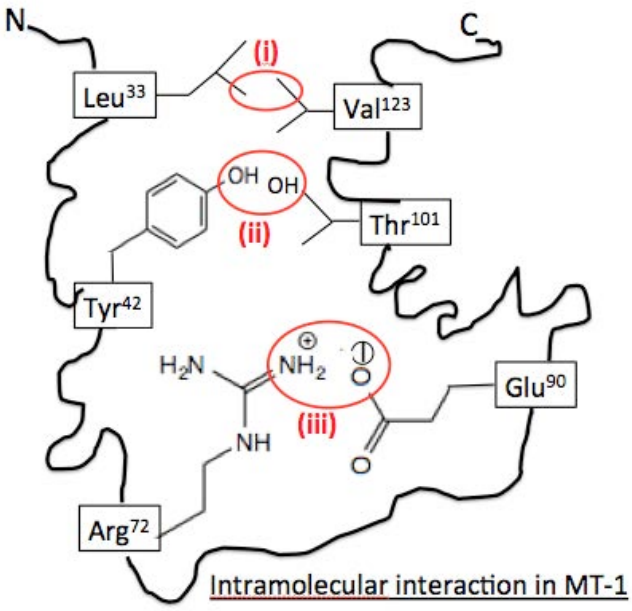
iv. In the sequence above, name the amino acid(s) that is…
Hydrophilic: *Thr* **Hydrophobic**: *Met, Ile, Ala*

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.



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

i. ***Hydrophobic interaction***
ii. ***Hydrogen bonding***
iii. ***Ionic interaction***

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. **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.

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)

*The MT_1 in **Individual A** will be able to bind to melatonin. The amino acids (Ile^{33} & Ala^{123}) have hydrophobic side-chains, which are approximately of the same size as the original amino acid (Leu^{33} & Val^{123}) allowing for hydrophobic interaction at position (i). So the 3D conformation of MT_1 is maintained that allows it to bind to melatonin. Similarly, the amino acid substitutions in **Individual B** allows for the formation of a hydrogen bond at position (ii). So MT_1 will maintain its 3D conformation and bind to melatonin. In **Individual C**, although both Val and Trp have hydrophobic side-chains, the side-chain of Trp is significantly bulkier than that of Val, this will create steric hindrance thus destabilizing the MT_1 structure and preventing its binding to melatonin. The Lys in **Individual D** has a positively charged side-chain, which will repel Arg^{72} at position (iii) thus destabilizing the 3D- conformation of MT_1 and preventing its binding to melatonin.*

c) Based on the mutations in the table above, which individuals (A-D) will have insomnia and **why**?
The MT_1 in Individuals C and D from the table in part (b) is not able to bind to melatonin and promote sleep. Therefore these individuals will have a disrupted sleep cycle or show the symptoms associated with wakefulness or insomnia.

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.
The remaining amino acids allow the MT_1 protein to fold and acquire the right 3D- conformation, which is critical for the amino acids from different parts of the MT_1 protein to come together to form its substrate binding site.

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**?

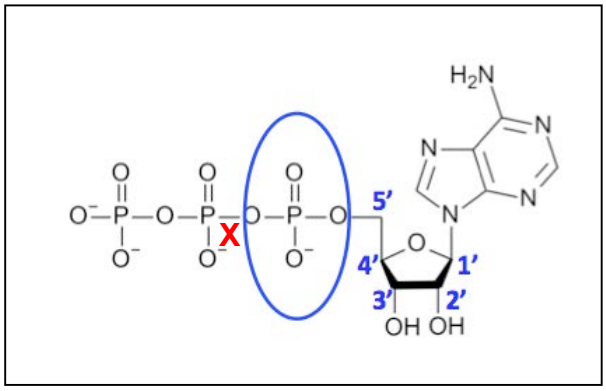
Although both MT-1 and MT-2 are of the same length (in terms of base pairs), MT-2 has a higher A/T% and therefore fewer hydrogen bonds compared to MT-1 which is rich in %G/C. So the energy needed to break the fewer hydrogen bonds and denature MT-2 sequence would be less than that needed to denature MT-1 i.e. MT-2 will denature at a lower temperature.

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

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

DNA duplex is likely to renature unlike most proteins. The bases in one DNA strand can hydrogen bond with the complementary bases in the other strand. This makes the denaturation of dsDNA reversible. In comparison proteins usually fold/ aggregate to form complex 3D structure that is stabilized by different types non-covalent interactions (ionic/ hydrogen bonding/ hydrophobic/ VDW) and covalent bonds (S-S) so that they acquire a stable, low energy state. Once denatured, reforming this complex 3D conformation is almost impossible.

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**?

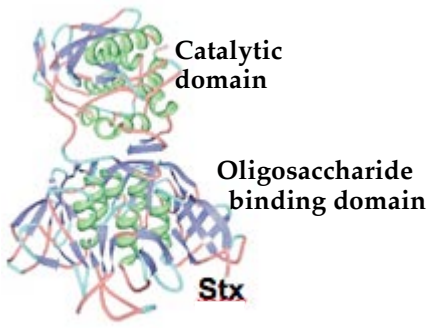
This ATP can be a part of RNA since it has a ribose sugar with a 2'-OH group. The nucleotides that make the DNA have deoxyribose sugar with an "H" at the 2'C position.

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.

It is important to note that the addition of an incoming base to the 3'OH end of a growing nucleic acid change is an energy requiring reaction. This energy is derived by the hydrolysis of the bond (shown as X), which results in the formation of pyrophosphate (PPi) and AMP. It is the AMP, which then gets added to the 3' OH end of nucleic acid chain.

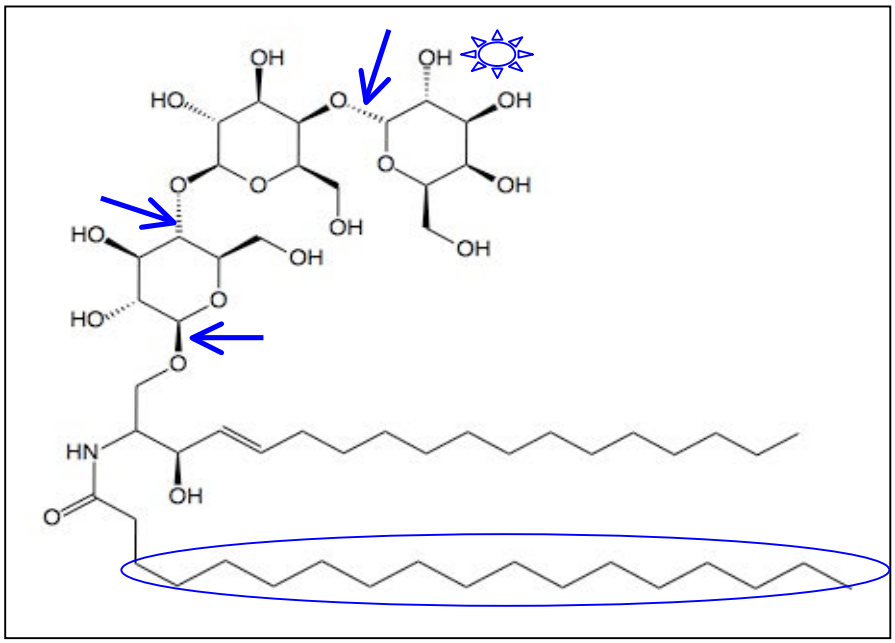
Question 6
Many drugs that promote sleep can cause diarrhea as a side effect.

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.

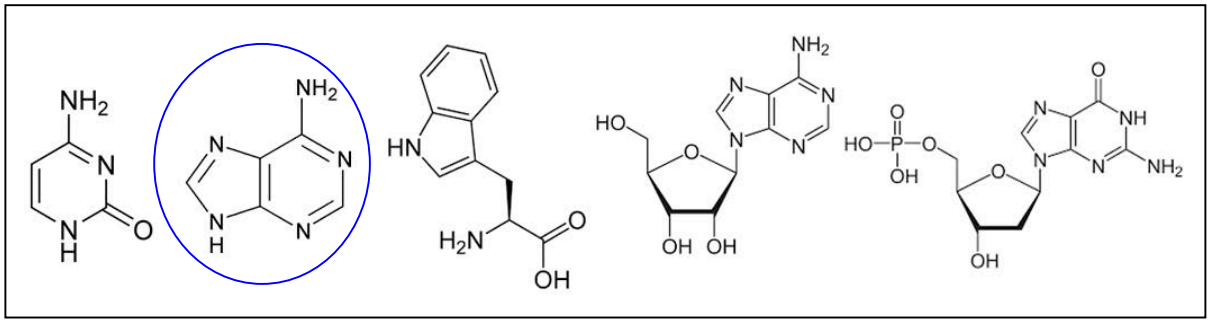
iii. Indicate the hydrophilic end of Gb3 with a **star**.

b) Complete the sentence. The oligosaccharide-binding domain of Stx is mostly composed of ***β-sheets***, which are stabilized by ***hydrogen bonds*** 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.*
You can list any amino acid with hydrophilic side-chain since they can form hydrogen bonds. Alternatively, you can list any hydrophobic amino acid and explain that these undergo hydrophobic interactions with the C_H bonds of the sugar rings.

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**? *Bacteria and skin cells*

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

Mitochondria are the ready source of energy (remember the powerhouse of the cell!). Mitochondria have a double membrane and they have a circular genome just like bacteria. This reflects that they arose from symbiosis where one small cell was engulfed by a bigger cell and both cells persisted in a symbiotic relationship. Mitochondria also have their own ribosomes, which are similar to bacterial ribosomes. Furthermore, the size of a mitochondrion is almost the same as the size of bacteria.

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	*Mitochondria*
Proteins that participates in transcription of genes	*Nucleus*
Proteins that is involved in digesting toxic material in the cell	*Lysosomes*
Proteins that adhere identical eukaryotic cells together and participate in intercellular (cell to cell) communication	*Plasma membrane*
Proteins that are a part of a dynamic network of fibers that maintain the shape and motility of a eukaryotic cell	*Cytoskeleton*
Enzymes that are involved in modification of newly synthesized proteins	*Golgi and ER*

7.013 Introductory Biology

Spring 2018