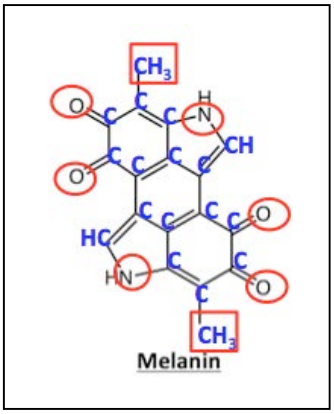
Solution key- 7.016 Problem Set 1- 2018

Question 1 (3pts)

The following is the "line-angle" drawing of melanin, a pigment that determines hair color. **Note:** *The carbon (C) and the hydrogen (H) atoms are not shown but implied.*



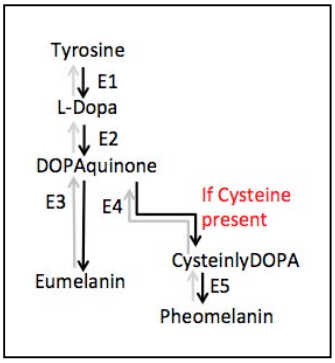
a) Clearly label **ALL** C and H atoms on the line angle drawing and write the **molecular formula** of melanin in the space below. $C_{18}H_{10}O_4N_2$ *(0.5)*

b) On the line angle drawing, **box one** nonpolar functional group *(0.5)* and **circle all** electronegative elements *(0.5)*.

c) Do you think melanin would dissolve in water? **Why or why not?** *Melanin has multiple electronegative elements (circled in the schematic) that can hydrogen bond with the surrounding water molecules allowing it to dissolve in water. You may also argue that although melanin has multiple electronegative elements, it has bulky aromatic rings and carbonyl group, which makes it a weak organic acid that does not dissolve in water. (These organic molecules are usually soluble in alkaline solution or solvents such as dimethyl sulphoxide or DMSO). (1pt, only the explanation will be graded)*

Question 2 (3pts)

There are two types of melanin pigment in hair follicles: **pheomelanin** (which promotes red or blond hair color) and **eumelanin** (which promotes black or brown hair color). The following is the simplified outline of eumelanin and pheomelanin synthesis.



a) The E1-E5 catalyzed reactions proceed spontaneously in the forward direction (shown by an →) and not in the reverse direction (shown by ↛) within a cell. **Explain** why this is so.

There are multiple correct answers: E1-E5 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. (1pt, only one reason enough for full points)

b) You identify three individuals: **Individual A** lacks a functional E2, **Individual B** has a hyperactive form of E3 enzyme and **Individual C** has a functional E5 but lacks a functional E4.

i. Which metabolite(s) would build up in the melanin synthesizing cells of **Individual A**?

L- Dopa (and Tyrosine) (0.5, L-Dopa with or without Tyrosine is ok for full points)

ii. Which metabolite(s) would build up in the melanin synthesizing cells of **Individual B**?

Eumelanin (0.5)

iii. What would be the hair color of Individual C? **Explain** your choice assuming that cysteine is present. *In the absence of a functional E4, DOPAquinone→ CysteinlyDOPA reaction step would not proceed even in the presence of Cysteine. So no pheomelanin will be synthesized. Instead Dopaquinone will continually be converted to eumelanin by E3 catalyzed reaction resulting in black or brown hair color. (0.5, only explanation will be graded)*

Question 2 continued

c) Which class of macromolecules is cysteine a monomer of: **carbohydrates/ lipids/ nucleic acids/ proteins**? Briefly **explain** why cysteine is different from other monomers that make the class of macromolecules that you chose.

This is the only amino acid with a side-chain $-CH_2SH$ that allows it to form a disulphide (covalent) bond with another cysteine. (0.5)

Question 3 (5pts)

a) Complete the statements below by choosing from the following: **the same/ higher/ lower.** The reaction catalyzed by a hypoactive form of E3 (1000- fold less active than the normal form of E3) has …

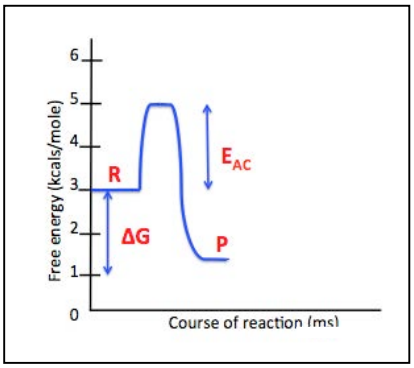
i. ***The same*** **free energy** change as the reaction catalyzed by normal E3**.** *(0.5)*

ii. ***Lower*** **reaction rate** compared to the reaction catalyzed by normal E3. *(0.5)*

iii. ***The same*** **reaction equilibrium** compared to the reaction catalyzed by normal E3. *(0.5)*

iv. ***Higher*** **activation energy** compared to the reaction catalyzed by normal E3. *(0.5)*

b) For the E3 catalyzed step, the free energy change (**ΔG) = -1.8 kcals/mole**.



i. The E3 catalyzed reaction is an example of an **exergonic/ endergonic** reaction. *(0.5)*

ii. On the left, draw the energy profile of the reaction catalyzed by E3. Label the reactants (R), products (P), ΔG and activation energy (E_{AC}) of the reaction. *(0.5 for graph, 0.5 for labels)*

c) E3 is optimally active at pH 7.4 and 37^{O}C. If the same E3-catalyzed reaction were 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 melanin synthesis? **Explain why. Note:** *Provide an explanation with respect to the three dimensional (3D-) conformation of E3 enzyme. Your explanations may vary.*

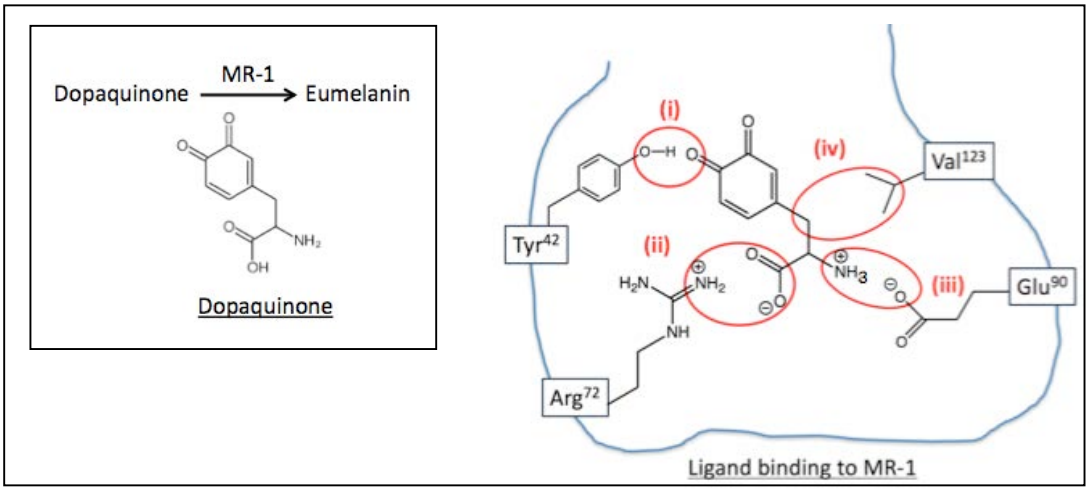
It is possible that a change in the temperature would denature the enzyme. In the absence of the enzyme the activation energy will not be lowered thus reaction will either not proceed or will proceed at a very slow rate. You can also argue that an increase in temperature may increase the random movement of the substrate ($\Delta G = \Delta H - T\Delta S$) thus reducing the free energy. This may favor the reaction progress (but this in a biological system is less likely). (0.5, with 0.25 for explanation)

d) 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 active site of E3 and prevents the binding of the substrate to E3. 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. Drug B on the other hand competes with the substrate to bind to the active site of the enzyme. The effect of Drug B depends on its concentration relative to the substrate and whether it binding to the active site of E3 is covalent (irreversible) or non-covalent (reversible). This makes Drug B a competitive inhibitor. (1pt, with 0.5 for explanation)

Question 4 (5pts)
E3 (or the Melanocortin receptor (MR-1)) catalyzes the conversion of dopaquinone to eumelanin as shown below. ***Note:*** *Each circled interaction is critical for dopaquinone-MR-1 binding.*

a) For each position (i)–(iv), name the **non-covalent interactions** between MR-1 receptor and dopaquinone by choosing from ***ionic/ hydrogen/ hydrophobic interactions.***



(i): ***Hydrogen****(0.5)* **(ii):** ***Ionic****(0.5)* **(iii):** ***Ionic****(0.5)* **(iv):** ***Hydrophobic*** *(0.5)*

b) You identify four individuals (1-4), each having an amino acid substitution in the MR-1 protein at the positions outlined below.

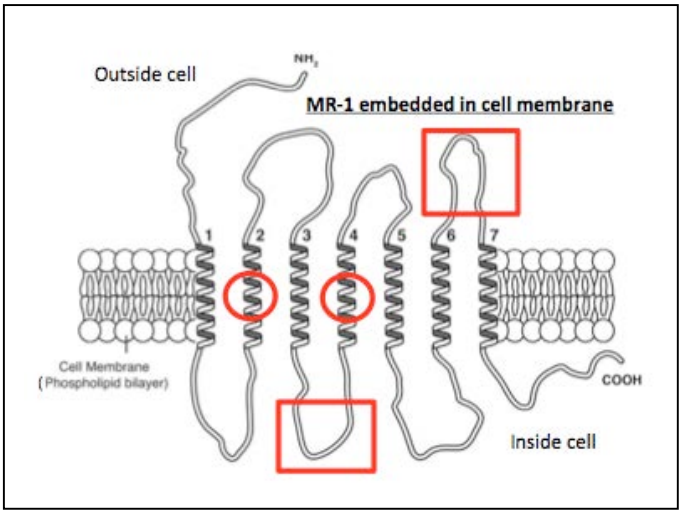
Individuals	Amino acid substitutions
1	Tyr^{42} → Ile^{42} at position (i)
2	Arg^{72} -> Asp^{72} at position (ii)
3	Glu^{90} -> Asp^{90} at position (iii)
4	Val^{123}→ Ala^{123} at position (iv)

Explain, in terms of the type of mutation what would be the likely hair color for Individuals 1-4. *The MR-1 in* ***Individual 1*** *is non-functional. The amino acids (Ile^{42}) at position (i) has hydrophobic side-chains and can therefore not form at hydrogen bond unlike Tyr^{42}. In the absence of functional E3 / MR-1 the person will only make pheomelanin and will have red or blond hair. The Asp in* ***Individual 2*** *has a negatively charged side-chain, which will repel Dopaquinone at position (ii) thus destabilizing the 3D- conformation of E3/ MR-1 resulting in pheomelanin synthesis, which accounts for the red or blond hair color.*

Both Glu and Asp at Position (iii) have hydrophilic side-chains of approximately the same size. So they can form ionic bond. So ***Individual 3*** *can synthesize eumelanin and will therefore have black or brown hair. In* ***Individual 4****, both Val and Ala at position (iv) have hydrophobic side-chains So E3/ MR-1 will be likely active resulting in black or brown hair in Individual 4. (2pts, with 0.5 for explanation related to each Individual)*

c) Although the active site of MR-1 is composed of only a few amino acids, the MR-1 protein as a whole is composed of many amino acids. **Explain** how the amino acids outside of the active site of MR-1 may contribute to its function.
The remaining amino acids allow the MR-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. (1pt)

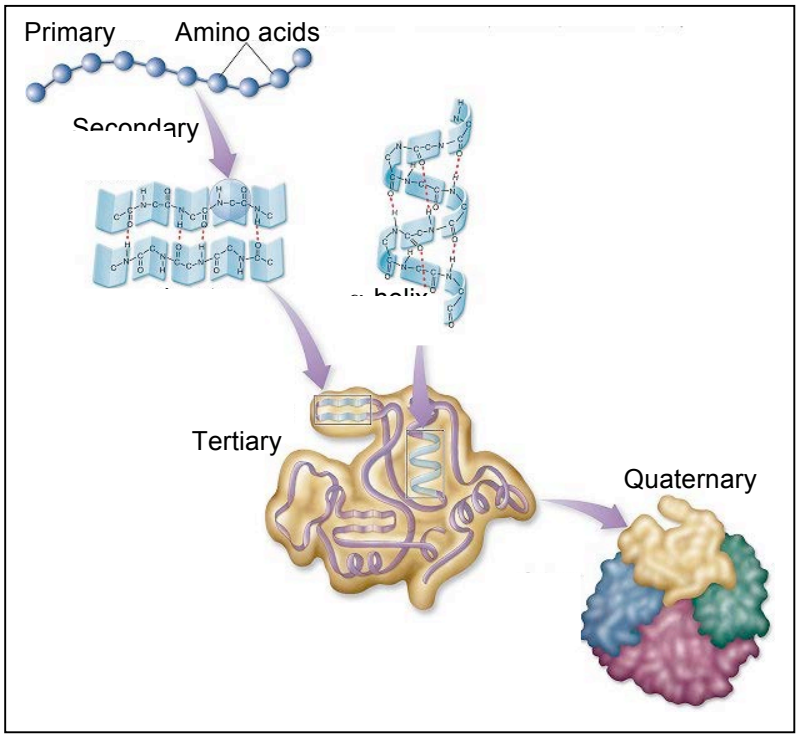
Question 5 (4pts)
The schematic below shows MR-1 receptor embedded in the cell membrane. This receptor has 7 transmembrane domains labeled 1-7 in the schematic.



a) Does the nature/ characteristics of amino acids inside **the circled region** of MR-1 differ from the nature of amino acids inside **the boxed regions**? If so, **why**?
MR-1 is a transmembrane protein since it spans the plasma membrane. The amino acids of MR-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 MR-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. (0.5)

b) Would the sequence of amino acids in both circled regions ALWAYS be the same? **Why or why not?** *No, the nature of their side-chain is likely to be the same (hydrophobic) so that the transmembrane domains are compatible to the hydrophobic interior of the lipid bilayer. But these hydrophobic amino acids (AVILMFYWPG) can be arranged in different permutations and combinations within the primary structure of MR-1. (0.5)*

c) The following schematic depicts different levels of structure for the MR-1 receptor that functions at pH 7.4. Answer the questions below by choosing from: **primary/ secondary/ tertiary/ quaternary.** Select **All** that apply**.** *(1pt or 0.25 each)*



i. Which level of MR-1 structure is stabilized only by hydrogen bonding? *Secondary*

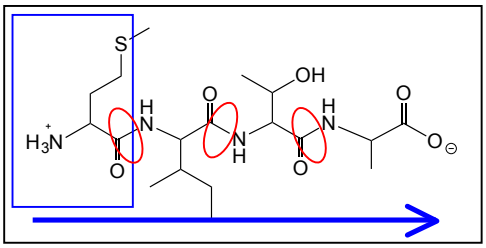
ii. Which level of MR-1 structure shows only the covalent amide/peptide bonds? *Primary*

iii. If MR-1 is exposed to an acidic pH, which level of protein structure **remains unchanged** even when the protein is denatured? *Primary*

iv. Which level(s) of MR-1 structure is stabilized by **INTRA** molecular non-covalent interactions? *Tertiary and secondary (due to the hydrogen bonds between the C=O and NH groups of the peptide chain backbone)*

Question 5 continued

d) The first four amino acids of MR-1 are shown in the diagram below.



i. On the diagram, show the direction of synthesis of MR-1 by an arrow and box the **first amino acid**. *(0.25 for direction and 0.25 for 1st amino acid)*

ii. On the diagram, circle **ALL** the peptide bonds between the amino acids. *(0.25)*

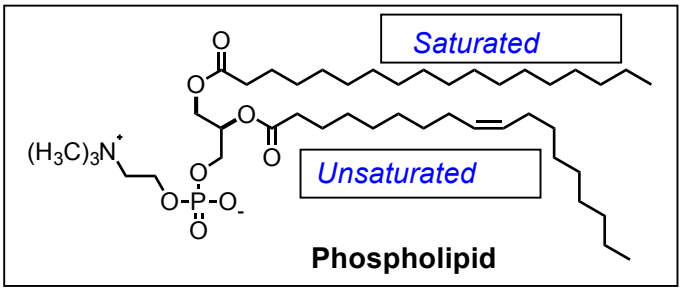
iii. Give the **byproduct** of a peptide (amide) bond synthesis reaction and classify the reaction as **condensation** or **hydrolysis**. *Water (0.25)*

iv. Which amino acid(s) in the sequence above is **hydrophilic**? ***Note:*** *An amino acid table is provided on the last page of this problem set.* *Threonine/ Thr/ T (0.25)*

v. Which of the following did you consider while answering part (iv) above? *(0.25)*

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

e) MR-1 is localized to and functions in the cell membrane. This membrane is made up of phospholipid molecules.



i. On the schematic, identify the saturated and unsaturated fatty acid chains by filling in the boxes. *(0.25)*

ii. **Circle** the best option: The phospholipids that make up the plasma membrane are **hydrophilic/ hydrophobic/ amphipathic.** How does your choice allow them to arrange to form the lipid bilayer in water?

The amphipathic nature (polar phosphate heads that face the outside and the inside of the cell & nonpolar hydrocarbon chains that face each other and are away from the aqueous environment) of these molecules allows them to assemble and form a lipid bilayer. (0.25, if correct explanation)

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