BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI
FIRST SEMESTER 2023-24
BIO F213 CELL BIOLOGY – Mid-Semester Examination
DATE: 13.10.2023 M. Marks: 50 Maximum Duration: 90mins

Q1. A researcher is interested in investigating the fluidity of the mitochondrial membrane. Hence, she tagged a mitochondria-localized membrane protein with a fluorescent protein. Fluorescence microscopy in living cells has revealed that the protein is indeed localized in the membrane of mitochondria.

a. What technique the researcher would employ to investigate the fluidity of the mitochondrial membrane? **2M**
b. How would the researcher interpret the results (i.e., degree of fluidity) obtained from the technique that she has employed for her investigation? **2M**
c. She has found that the mitochondrial membrane that she is investigating is less fluidic, and as a result, the movement of the proteins from the cytoplasm into mitochondria is also affected. Justify, how both phenomena are interconnected. **3M**

Q2. What would be the specific consequence of the following mutations in a cell? Provide your answers with appropriate molecular mechanisms. **4x2.5 = 10M**

a. Mutation leading to inactive dynamins
b. Mutation leading to the absence of v-SNARE proteins in the synaptic vesicle in a neuron
c. Removal of the ER signal sequence from a protein
d. Accumulation of misfolded proteins in the Rough Endoplasmic Reticulum (RER)

Q3. After the digestion of food material in your gut, the concentration of glucose that is present in the gut lumen is usually low compared to the epidermal cells lining the intestine.

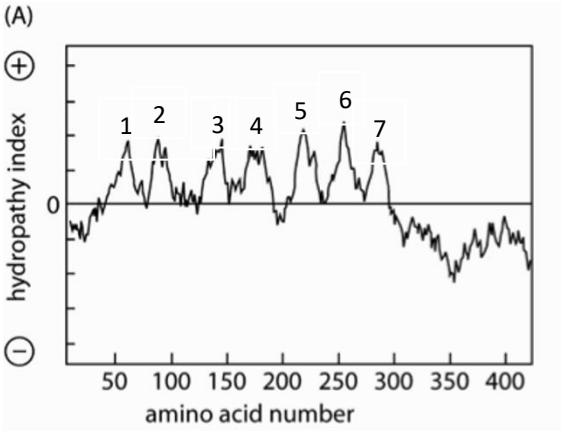
a. What is the efficient strategy that the cells would employ to transport the glucose from the gut lumen to the cytosol of the intestinal cell? Briefly explain the transport mechanism. **3M**
b. The process of transportation of glucose inside the cells generally leads to the disturbance in certain ion concentrations. How would the cell maintain the ion concentrations after the import of glucose molecules? Briefly explain the mechanism that is involved in this process. **3M**

Q4. After extensive practical and theoretical training in microscopy, you are interested in observing the following in an isolated cell. Which microscopic approach you would employ to visualize the sample in each of the cases given below? Justify your answer briefly in each case.

a. Specific detection of an ATP synthase complex located in the mitochondrial membrane **3M**
b. Monitoring the movement of parasites in blood without using any stains or dyes **3M**

Q5. During protein synthesis in the Rough Endoplasmic Reticulum (RER), a 14-sugar block that is already preassembled is added to the conserved asparagine residue of the protein. However, the 14-sugar block is further remodeled in the Golgi apparatus leading to a completely different structure before they reach the destination. Explain why is it important for a cell to add the preassembled 14-sugar block to the protein and then process them to make different sugar structures in the end. Also, comment on the functional significance of the N-glycosylation process that occurs in the RER. **4M**

Q6. Below is the hydropathy plot of a membrane protein that is tagged with a green fluorescent protein (GFP). The protein is synthesized in the Rough Endoplasmic Reticulum (ER) and the final destination of the protein is the plasma membrane of the cell. The protein carries several START transfer and STOP transfer sequences as shown below. 1 = N-terminal non-cleavable START transfer sequence. 2,4,6 = STOP transfer sequences. 3,5,7 = START transfer sequences. The last START transfer sequence (7) is followed by a non-cleavable GFP tag.



a. Based on the plot and the given information what is the secondary structure in this protein that is responsible for their association with the membrane protein? Justify **2M**
b. Can this protein be isolated from the membrane by gentle extraction? Justify **3M**
c. Predict the orientation of the GFP inside the vesicle that has budded off from the Golgi apparatus before integrated into the plasma membrane. Justify your answer with a diagram **3M**

Q7. A researcher is investigating two transcription factors, A and B, that regulate the expression of genes in the nucleus. Both transcription factors A and B carried a perfect nuclear-localized signal (NLS) sequence without any mutation for transport across the nucleus. She tagged both the proteins with GFP and expressed them in an animal cell line. During the investigation, she noticed that transcription factor B was transported into the nucleus and subsequently regulated the expression of its corresponding gene. However, transcription factor A was detected only in the cytoplasm.

a. What might be the reason for transcription factor A to remain in the cytoplasm albeit having the correct NLS sequence? Will this transcription factor A under any circumstances move inside the nucleus? Justify your answer **3M**
b. What will be the consequence of substituting a negative charge amino acid in the NLS region of the transcription factor B? **2M**
c. With the help of a diagram sketch the mechanistic details about how the transcription factor Y moves into the nucleus. 4**M**