Candidate number ……..…….……
Course code ……..
Page no……...

NORGES TEKNISK-NATURVITENSKAPELIGE UNIVERSITET
INSTITUTT FOR FYSIKK

EXAM IN TFY4260 – CELL BIOLOGY AND CELLULAR BIOPHYSICS

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Time: 09.00-13.00

All questions in Exercises 1 to 5 have the same weight (5 pts). Questions in Exercise 6 count with 1 pt each (15 in total). None of the questions requires lengthy answers so answer as precisely and concisely as possible.
Good luck!

Exercise 1: Lipid membranes and transport across membranes

a) True or false? Transport by transporters can be either active or facilitated, whereas transport by channels is always facilitated. Justify.
b) The intake of glucose into erythrocytes is done via glucose transporter GLUT1. Once inside the cell, the glucose is quickly phosphorylated to glucose-6-phophate. Why? (Hint: use transport arguments)
c) Order the following membranes in terms of protein/lipid ratio: rough endoplasmic reticulum (ER), smooth ER, inner mitochondrial membrane, and myelin sheath of nerve axon. Justify.
d) You are given a sample with unstained red blood cells in a solution. Which microscopy technique would you use to view the shape of the cells? Explain your choice.

Answers:

a) True. Transporters bind specific molecules and undergo a series of conformational changes to move the bound molecule across a membrane. They can transport passively down the electrochemical gradient (facilitated diffusion), but they can also undergo conformational changes when linked to a source of metabolic energy such as ATP hydrolysis, and drive active transport. Channels, on the other hand, form aqueous pores that can be open or shut but do not undergo conformational changes that allow molecules or ions to move against its electrochemical gradient. The transport through channels is always passive.
b) Glucose transporter GLUT1 is responsible for the passive diffusion of glucose into and out of erythrocytes, depending on its relative concentration in and out. The phosphorylation of glucose into glucose-6-phosphate inside the cells keeps the concentration of glucose low inside the cell, which makes facilitated diffusion possible. In addition the phosphorylation of glucose locks the molecule inside the cell, since the GLUT1 transporter does not recognized the phosphorylated form of glucose and has no specific transporter for it either. Phosphorylation is a general strategy for retaining molecules within the cells.
c) The order of increasing protein/lipid ratio is: myelin sheath of nerve axon < smooth ER < rough ER < inner mitochondrial membrane. The inner mitochondrial membrane is the

Candidate number ……..…….……
Course code ……..
Page no……...

membrane with the largest proteins to lipid ratio. Here ATP is produce by the movement of protons down their electrochemical gradient through ATP synthases so it is not surprising that these proteins are especially, in this membrane, which in addition has a large surface area, due to the presence of cristae. Next we would find the ER membranes, with the rough ER presenting a relatively larger concentration of proteins at the membrane due to the presence of the ribosomes. Finally the myelin sheath of nerve axons comes as having the lowest protein/lipid ratio of the four with only ~0.2. The function of these membranes is that of isolating segments of the axon, to speed up the nervous impulses and so the presence of proteins is less required in this case.

d) Phase contrast or differential interference contrast (DIC). In these techniques the contrast arises from the difference (phase contrast) or the gradient (DIC) in the optical path length. This gives high contrast to the cell membrane and allows the shape of the cell to be easily viewed.

Exercise 2: Cytoskeleton and contractibility

a) True or false? In most animal cells, motor protein dyneins deliver their cargo to the periphery of the cell, whereas kinesins deliver their cargo to the interior of the cell. Justify.
b) Most of the transfer of proteins and lipids is done with the so-called "coated vesicles". Why have the vesicles such name? Name **two** reasons for the presence of such structure.
c) Why is it that intermediate filaments lack polarity whereas actin filaments and microtubules have two distinct ends with a defined polarity?
d) True or false? Motor neurons trigger action potentials in muscle cell membranes that open voltage-sensitive Ca^{2+} channels in T tubules, allowing the extracellular Ca^{2+} to enter the cytosol, bind to troponin C, and initiate muscle contraction. Justify.

Answers:

a) False. In most animal cells the microtubules spring from microtubules organizing centers (MTOCs) with the 'plus' ends directed towards the periphery of the cell. Motor proteins that are associated with microtubules (MTs) move in specific directions. Kinesis are the motor proteins responsible for transport to the 'plus'-end of the MTs while dynein does the transport to the 'minus' end. So the correct answered would have been "In most animal cells, motor protein kinesins deliver their cargo to the periphery of the cell, whereas dyneins deliver their cargo to the interior of the cell.
b) The vesicles have such name because they are covered with a protein coat. The protein coat is believed to have a number of functions: (i) The type of coat on a vesicle helps in the sorting of molecules that are fated to different destinations; (ii) The assembly of the coat is believed to aid in the process of vesicles formation, by providing the system with the needed "energy" to overcome the unfavorable curvature that the lipid membrane must adopt to form vesicles. In addition, (iii) it prevents the premature and non-specific fusion of a forming vesicle with other membranes, and (iv) it regulates the interaction with the MTs that are important for the transport of the vesicles.
   Note: as requested, two of these (or other reasonable suggestions) were sufficient.
c) The (structural) polarity of the MTs and MFs come from the fact that the monomers they are composed of (tubulin and G-actin, respectively) are not symmetric and are oriented in the same direction within the protofilament and F-actin, repectively. This gives the fibers and inherent chemical polarity, commonly designated as '+' and '-'-ends. Not to be confused with "electrostatic type of polarity". The intermediate filaments are also constituted of proteins that are asymmetric but, in this case, they are aligned in parallel, in

Candidate number ……..……
Course code ……..
Page no……...

bundles of two dimers, with the N- and C- terminals pointing in opposite directions. Sequences of such tetramers form protofilaments that have identical chemical structure in the end and therefore possess no polarity.

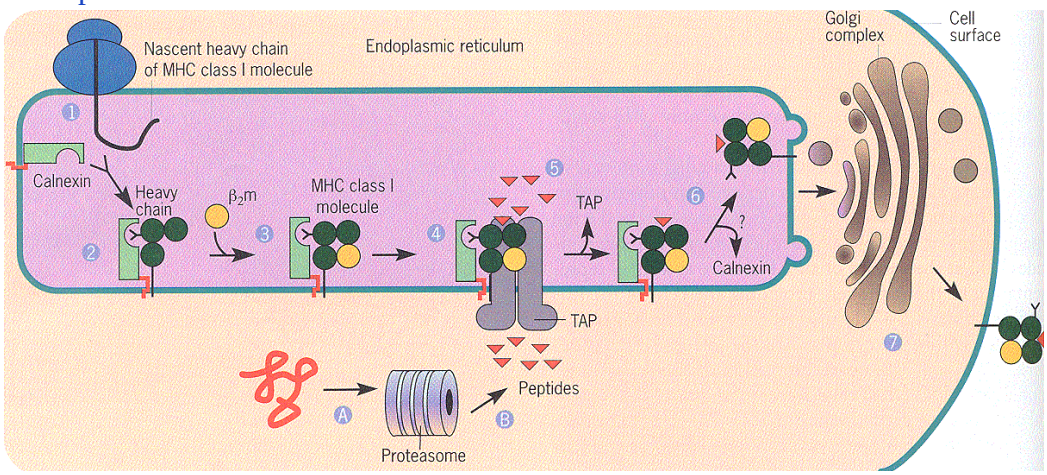
d) False. When motor neurons trigger action potentials in muscle cell membranes, these will open voltage-sensitive Ca^{2+} channels in the sarcoplasmic reticulum (SR) and not in the T tubules, as mentioned. So, the Ca^{2+} that binds to troponin C and allows the initiation of muscle contraction does not come from outside the cell, as mentioned but from the SR.

Exercise 3: Immunology and membrane-bound compartments

a) Explain the mechanism that regulates the selection of appropriate T-cells in the thymus.
b) After having been presented with antigen, B cells proliferate and differentiate into memory B cells and plasma cells. What are the most distinct structural and functional differences between memory and plasma cells? Justify.
c) Draw a rough scheme of the synthesis, processing and assembly of the class I MHC (major histocompatibility complex) – peptide (antigen) complex and antigen presentation, highlighting the most important points of this process.

Answers:

a) The selection of T-cell is very important for the organism to the able to distinguish between the self and non-self. The selection is, as mentioned, done in the thymus. It is here that the T-cells gain identity, that is, they undergo DNA rearrangements that enable it to produce a specific T-cell receptor (TCR). The cell is then subjected to a complex screening process, where it is presented to thymic cells with MHC proteins attached to peptides. The T-cells whose TCR show a high affinity for the thymic cells possessing own-peptides are destroyed (negative selection). In addition, T-cells whose TCRs do not recognize their own MHC molecules are destroyed (death by neglect), since these would be useless in case of an infection. So, only the cells whose TCRs exhibit weak affinity recognition towards self-MHC – self-peptide complexes are stimulated to remain alive (positive selection).
b) When a B cell binds to an antigen it proliferates and differentiates into memory B cells and plasma cells. The former may persist for a person's lifetime and can respond rapidly at a later stage if the antigen reappears in the body. Plasma cells' function is to produce soluble antibody molecules for secretion. These are synthesized in the rough ER, so these cells present a much larger cytoplasmic compartment than the B cell, with more mitochondria and an extensively developed rough ER.
c) Example of a scheme:



Candidate number ……..…….……
Course code ……..
Page no……...

There was no need to point out everything is detail. The important points of this process are (i) the synthesis of the MHC molecule is performed in the rough ER, since it is a transmembrane protein. As opposed to class II MHC (that present antigen fragments present in lysosomes), (ii) peptides are present in the cytosol of the cell, after digestion by proteasomes. (iii) Peptides are transported into the ER, with the aid of a transmembrane protein, where (iv) they bind the MHC molecule. The (v) complex passes through the Golgi complex for further processing and ends up at the plasma membrane, (vi) facing the outside of the cell.

Exercise 4: Cell signaling and cancer cells

Rous sarcoma virus (RSV) carries an oncogene called *Src*, which encodes a continuously activated protein tyrosine kinase (Src) that leads to unchecked cell proliferation. Normally, Src carries an attached fatty acid (hydrocarbon) chain that allows it to bind to the cytoplasmic side of the plasma membrane. A mutant version of Src does not possess the hydrocarbon chain. Infection of cells with RSV encoding either the normal or the mutant Src leads to the same high level of protein kinase activity, but the mutant Src does not cause cell proliferation.

a) What is it meant with continuously activated? Taking into account the general structure of receptor, give an example on how this could be achieved.
b) The target (X) for phosphorylation by Src resides in the membrane. Explain why the mutant Src does not cause cell proliferation.
c) Name **two** characteristics that cancer cells possess which contribute to the invasion and metastases of cancer cells.

Answers:

a) With continuously activated is meant that the receptor never switches off and is constantly signaling for, in the case of tyrosine kinase, cell proliferation. The tryrosine kinase receptor is activated by dimerization or clustering, in the presence of a growth factor (ligand). If two receptors are permanently bound, then they will be activated all the time, independently of the presence of not of growth factors.
b) The mutant Src does not possess the fatty acid chain and therefore instead of being attached to the cytoplasmic side of the plasma membrane, it will be soluble in the cytosol. Assuming that the concentration of the mutant Src is constant throughout the cell, this means that the concentration of Src by the membrane, where the secondary messenger for that particular signal transduction is, is much lower, when compared with that of normal membrane-bounded Src. Therefore, despite having the same high level of activity, mutant kinase receptors will not lead to a significant cell proliferation.
c) Cellular changes that contribute to invasion and metastases of cancer cells are (i) lack of cell adhesion transmembrane proteins (such as cadherins), which reduce the binding to other molecules, (ii) increased motility stimulated by signal (chemo-attractive) molecules secreted from tumor cells or surrounding normal cells, and (iii) secretion of proteases that induce the degradation of the extracellular matrix in connective tissue and of the basal lamina.

Exercise 5: DNA recombination and regulation of gene expression

In the absence of glucose *E. coli* can proliferate on pentose sugar arabinose, using an inducible operon called *ara*. The *araC* gene encodes a gene regulatory protein that binds adjacent to the

Candidate number ……..…….……
Course code ……..
Page no……...

promoter and coordinates the expression of the genes involved in the arabinose metabolism. To understand the regulatory properties of the AraC protein, you isolate a mutant bacterium with a deletion of the *araC* gene. The table below shows the gene expression of araA protein in the presence and absence of arabinose, for both bacteria phenotypes:

	araA protein	
	- arabinose	+ arabinose
wild type	1	1000
mutant	1	1

a) Do the results in the table indicate that the AraC protein regulates arabinose metabolism by negative control or by positive control? Justify. What would the data in the table indicate if the **AraA** protein was regulated by the mechanism you did not choose in a)?
b) What is an operon? Why are these less common in eukaryotes?
c) The first step in a DNA cloning procedure using bacteria is to insert the desired piece of DNA into an appropriate cloning vector. *pUC*19 is a popular plasmid cloning vector. It possesses an amp^R gene and 11 different restriction sites clustered in a region containing the *lacZ* gene, which codes for the enzyme β-galactosidase. Why are these features desirable?

Answers:

a) It is mentioned in the question that this is an inducible operon, meaning that the presence of an inducer (arabinose, in this case) activates the synthesis of araA protein, as seen in the table, for the wild type. Now, the regulation of an inducible operon can be done via negative control, where the arabinose binding to the araC regulatory protein leads to the switching on of transcription. This happens when araC protein blocks the binding of the polymerase to DNA or the progression of the polymerase along the DNA. The regulation can also be done via positive control, where the binding of the inducer to araC leads to an increase in gene expression. This would be the case if the araC – arabinose complex would improve the binding of the polymerase to the DNA, for example. With the data in the table we can see that the absence of *araC* gene, and therefore the absence of araC protein, leads to the depression of araA synthesis even in the presence of arabinose. This indicates that araC regulates the arabinose metabolism by positive control. If the control is a negative one, then the lack of araC protein would lead to the synthesis of araA protein, in the presence or absence of arabinose:

	araA protein	
	- arabinose	+ arabinose
wild type	1	1000
mutant	1000	1000

b) An operon a segment of DNA where genes with metabolically related functions are clustered together. This was their transcription can be regulated as a single unit. These are rather common in prokaryotic cells but are less common in eukaryotes. A reason for this is that eukaryotes synthesize a larger number of polypeptides than the number of genes present in their genome. Since different polypeptides can be synthesized from the same pre-mRNA molecule, these have to go through extensive post-transcriptional processing, and it makes little sense to cluster genes in this case. In eukaryotic cells, the expression of groups of related genes is done via transcription factors and response elements.

Candidate number ……..…….……
Course code ……..
Page no……...

c) When cloning a DNA sequence one wants to be sure that one is cloning the wanted sequence. The mentioned features of the *pUC*19 plasmid allow the selection of bacteria with the DNA of interest. The presence of the amp^R gene confers resistance towards antibiotics and so, only bacteria possessing the pUC19 plasmid will proliferate. The large number of restriction sites indicates that many different sequences of interest can be added to the plasmid. Recall that in order to add the DNA sequence to the plasmid both plasmid and the initial DNA molecule where the sequence of interest is extracted need to be cut using the same class of restriction enzymes. Finally, the fact that these restriction sites are present in a region containing the *lacZ* gene means that one is able to detect if the bacteria contain the initial plasmid or the recombinant vector. This is done by checking the presence of β-galactosidase.
Of course, it can happen that the piece of DNA that was introduced in the plasmid is not that of interest. This is typically verified by isolating the DNA, using restriction enzymes to confirm that the DNA has the expected pattern, and by sequencing it.

Exercise 6: Mark the correct alternative with a cross. Deliver these pages together with the answers of the other exercises. Do not forget to indicate the candidate number.

a) The Na+/K+ pump, responsible for maintaining electrochemical ion gradients is an example of
X direct active transport.
- indirect active transport.
- facilitated diffusion.

b) Transport of enzymes from the Golgi complex to the endosomes is done via
- constitutive secretion.
- regulated secretion.
X anterograde transport.

c) Transport of proteases from the ER to the lysosomes relies on the fact that two compartments have different
X pH.
- temperature.
- size.

d) Fusion of vesicles with target membranes is mediated by
X SNARE proteins.
- dynamin.
- actin.

e) KDEL amino acid sequence tags polypeptides for
- destruction by proteasomes.
- transport to lysosomes.
X transport to the ER.

f) The resting membrane potential arises from
- large differences in ion composition inside and outside of the cell.
X small differences in the charge distribution inside and outside of the cell.
- variations of pH inside and outside of the cell.

Candidate number ……..…….……
Course code ……..
Page no……...

g) Release of neurotransmitters in the synaptic cleft and binding to receptor on a muscle cell is an example of
- autocrine signaling.
X paracrine signaling.
- endocrine signaling.

h) G protein-linked receptors activate a particular type of G protein (guanosine binding protein) that it turn activates other proteins, initiating a signaling pathway. The G protein is composed of three subunits (α, β, and γ), where subunits G_β and G_γ are permanently bound. Which subunits are able to initiate a signaling pathway?
- G_α.
- $G_{\beta\gamma}$.
X Both.

i) Microvilli from intestinal mucosa are cells are made of organized arrays of
- microtubules.
X microfilaments.
- intermediate filaments.

j) Integrins indirectly bind the extracellular matrix to
- microtubules.
X microfilaments.
- intermediate filaments.

k) In cell division, metaphase is characterized by the
- separation of the two sister chromatids of each chromosome.
- break down of the nuclear envelope.
X alignment of the chromosomes in the center of the cell.

l) The contractile ring formed during early anaphase, which leads to the cytoplasmic division of the cell is composed of
- tubulin.
X actin.
- dynamin.

m) In the cell cycle, Cdk-Cyclin regulates progression though the restriction point by phosphorylating the
- p53 protein.
X Rb protein.
- E2F transcription factor.

n) The p53 tumor suppressor gene is the most frequently mutated gene in human cancers. Which process is p53 involved in?
X Apoptosis.
- Angiogenesis.
- Sarcoplasmic reticulum.

Candidate number ……..…….……
Course code ……..
Page no……...

o) Telomere integrity in cancer cells is the result of
- natural cell aging.
- DNA replication.
X active telomerase activity.