MCB110
Third Midterm

May 10, 2010

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Your name and student ID

QUESTION	POINTS
1 (15 points)	
2 (15 points)	
3 (15 points)	
4 (20 points)	
5 (15 points)	
6 (15 points)	
7 (15 points)	
8 (25 points)	
9 (20 points)	
10 (20 points)	
11 (25 points)	

TOTAL (200 points)

WARNING: Your exam will be taken apart and each question graded separately. Therefore, if you do not put your name and ID# on every page or if you write an answer for one question on the backside of a page for a different question, you are in danger of irreversibly LOOSING POINTS!

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Q1 – What is a liposome? (5 pts) Describe one biochemical application (5 pts) and one medical application (5 pts), explaining, briefly but clearly, the benefits of using such system.

It is an artificially created spherical bilayer of lipids, a few nanometers in diameter, where the lipid composition is controlled and where proteins, molecules and chemical groups can be added at will. (5 pts)

It can be used to study reconstituted, purified integral membrane proteins, in a controlled, known lipid environment. (5pts)

It can be used as a drug delivery system, for either soluble molecules (in the lumen) or hydrophobic molecules (within its bilayer). Target elements can be placed on its surface, like antibodies, and it can be protected from attack by the immune system using polymer chemistry on its surface. (5 pts)

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Q2 – What is a lipid raft? Describe its essential physical property (5 pts). What types of lipids are present in rafts? (5 pts) What physiological role do they play in cells? (5 pts)

It is a region of a lipid bilayer in a gel state, distinct from the surrounding, fluid bilayer. (5 pts)

These regions are rich in cholesterol and sphingolipids. (5 pts)

Rafts typically house membrane proteins important in signaling processes. (5 pts)

Q3 – Why are integral membrane proteins difficult to study biochemically? Three reasons, explained, to get full credit (15 pts)

(1) They tend to be low abundance.
(2) Over expression in heterologous system is difficult and leads to inclusion bodies
(3) Purification requires solubilization with detergents. It is difficult to chose a detergent that will solubilize without affecting protein structure

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Q4 – A new unicellular organism has been discovered on the surface of Mars. The resting potential of its plasma membrane is defined by Ca^{2+} leaking channels, rather than K+. The calcium concentration is kept 10^3 times lower inside the cell than outside by means of a calcium ATPase pump. What is the resting electric potential across the plasma membrane of this organism? (Hint: because of the leaking channels, calcium is at equilibrium; no other ions are) (20 pts)

$\otimes G = 2.3RT \log 10\ [Ci\ ] / [Co] + zF \otimes E$

At equilibrium $\otimes G = 0$

$2.3RT \log 10\ [Ci\ ] / [Co] = - zF \otimes E$

$2.3RT \log 10\ 10^{-3} = - zF \otimes E$

$2.3RT\ (-3) = - zF \otimes E$

$1.4\ \text{kcal/mol}\ (-3) = - 2 \times 23.06 \otimes E$

$\otimes E = 4.2/46.12 = 0.091\ V$ *or* ***91 mV***

(or they can say ~ 100 mV if they do not have calculators for the last step. It is important that they get the sign right!! It is positive as opposed to the negative value in our cells with K leaking channels.

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Q5 – What is the function of the Na/K ATPase and why is it important physiologically for the cell? (5 pts) Describe the steps taking place during a cycle of ATP hydrolysis, concentrating on protein conformation and ion affinities, and their consequences (10 pts)

Its function is to generate ion gradients of sodium (low inside the cell) and potassium (high inside the cell). These are important to avoid osmotic shock, and to drive biological processes such as action potentials. (5 pts)

In its E1 state (unphosphorylated), the pump is open to the inside and has high affinity for sodium and ATP. ATP hydrolysis and phosphorylation of the pump leads to a conformational change into the E2 state, in which the pump is now open to the outside of the cell, looses affinity for sodium (which is released) and gain affinity for potassium, which binds. Upon dephosphorylation the pump reverses to the E1 state, opening to the inside and loosing affinity for potassium. The cycle then repeats. (10 pts)

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Q6 – How does the Na-glucose cotransporter make use of preexisting ion gradients? (5 pts) Place this cotransporter in the physiological context of the brush border cells of the small intestine: where is it present, what other transporters/ATPases are required for the proper functioning of these cells to provide glucose to the blood stream following digestion, and overall flow of glucose. (10 pts)

This transporter uses the preexisting sodium gradient to couple it to movement of glucose against a concentration gradient. Na moves down its electro-chemical gradient, from the outside to the inside, and glucose is moved against a concentration gradient, from the outside to the inside (symporter). (5 pts)

In brush border cells the Na-glucose symporter is in the apical membrane and allows the passage of glucose from the intestine into the cell. This glucose, which becomes very concentrated inside the cell, then leaves by passive diffusion, down its concentration gradient, via glucose transporters on the basal membrane, thus, reaching the blood stream for distribution to the rest of the body. The basal membrane also houses the Na-K ATPase that is required to maintain the Na gradient. (10 pts)

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Q7 – What is the unique property of voltage gated channels that makes action potentials move unidirectionally? (5 pts) What molecular element is responsible for this property (3 pts) and how was it tested experimentally (7 pts)?

They become desensitized soon after opening. (5 pts)

The existence of a "plug", a globular domain at the end of an unstructured region, that binds to the open channel leads to desensitization soon after channel opening. (3 pts)

This model was tested by measuring ion currents through voltage gates channels for the wild type protein, a mutant lacking the plug domain, and this mutant in the presence of the domain in trans, as a soluble peptide. While in the first there is a pulse of ion flow, the second leads to a jump in current that then is maintained due to the failure of the channel to be plugged. Addition of the peptide leads to a slower, but clear desensitization of the channel as seen by the decline in conduction. (7 pts)

Q8 – What is function of the SRP in the secretory pathway? (5 pts) What are the roles of GTP binding and GTP hydrolysis in the recruitment of active ribosomes to the ER for cotranslational translocation, and what triggers each one of them? (20 pts)

To bind to signal sequences on the nascent polypeptide channel, block translation elongation, and bring the stalled ribosome to the rough ER membrane. (5 pts)

GTP binding, which is triggered by interaction with the signal sequence, is required for the interaction of the SRP with its receptor on the ER membrane. (10 pts)
GTP hydrolysis, which occurs after SRP-receptor interaction, leads to the loss of affinity between them and the release of the SRP for another round of ribosome binding. (10 pts)

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Q9 – For the cellular functioning of coated vesicles, indicate in one or two short sentences what is the role of:

a) the cargo receptors (5 pts)

To bind both to proteins that need to be transported in the vesicle and to coat proteins, resulting in the concentration of the cargo molecule inside the forming vesicle

b) the GTPase (5 pts)

First, while in their GTP state, to nucleate the assembly of the vesicle by interacting with and bringing to the membrane the rest of the coat proteins. After vesicle budding, in its GDP state, to trigger the disassembly of the vesicle that is required prior to the fusion with the target membrane

c) the self-assembling coat proteins (5 pts)

Via interaction with cargo receptors, to enrich in cargo molecules, and by self-assembling, to produce mechanical force for the deformation of the vesicle that leads to budding

d) the v-snare protein (5 pts)

To target the uncoated vesicle to its target membrane via the specific interaction with its corresponding t-snare.

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Q10 – What are the respective roles of GAP and GEF proteins in the regulation of G proteins and their roles in signaling pathways (10 pts)? What are the roles of adaptor proteins in signaling events? Give an example of an adaptor in the activation of the MAP kinase cascade by EGF (10)

GAPs activate GTP hydrolysis leading to the deactivation of the G protein. (5 pts)

GEF promote the disassociation of GDP from the G protein, thus facilitating the binding of GTP, and therefore the activation of the G protein. (5 pts)

Adaptors serve as are multidomain proteins recruiting elements in signaling cascades. The example is Grb2, which interacts with phosphotyrosine motifs in activated receptor tyrosine kinases, and then binds and bring to the membrane Sos, which is a Ras GEF, leading to the activation of Ras and the consequent activation of the MAP kinase cascade. (10 pts)

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Q11 – Explain, in one or two sentences, how each of these essential steps in signaling are achieved in the case of the glucagon signaling in liver cells that results in glycogen breakdown:

- Initiation of the signaling process (5 pts)

  Binding of glucagon to its receptor on the plasma membrane, leads to a conformational change on the cytosolic surface of the receptor that then allows it to interact and activates a Gs.

- Activation of an effector (enzyme) protein that generates a secondary messenger (5 pts)

  Active Gs (bound to GTP) interacts with adenylyl cyclase, thus activating this enzyme, which produces cAMP

- Amplification of the signal (5 pts)

  The signal is amplified by having: 1) one receptor activating several Gs proteins; 2) an activated adenylyl cyclases producing many cAMPs; 3) a PKA phosphorylating many target proteins; 4) a phosphorylase kinase phosphorylating many phosphorylase molecules; 5) and a phosphorylase enzymatically hydrolyzing many bonds of the glycogen polymer.

- Shutdown of the signaling upon disappearance of glucagon (hint: consider all steps along the pathway in your answer)(10 pts)

  cAMP is constantly being broken down by phosphodiesterase. When glucagon disappears and its receptor becomes inactive, the Gs becomes deactivated and adenylyl cyclase stops producing cAMP. Thus the cAMP goes down. Inhibitor 1 becomes deactivated and phosphatase 1 in turns becomes active, leading to the dephosphorylation of the proteins involved in this cascade.