Exam: Cellular Chemistry, part Poolman (60 points)

Date: April 3, 2018
Time: 09:00-12:00 h
Location: 5118.-156

Name	. . . . .
Student number	. . . . .
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10 questions with sub-questions; the maximum score is 100 points and the final exam grade is the number of points divided by 10.

Please present your answers below the questions, and, if needed, please use the backside of the sheet rather than separate papers. The answers do not need to be long, and use scheme or drawing to illustrate your points.

Q1. Excluded volume interactions (10 pt)

Excluded volume interactions favour helix formation of a polymer, and in fact the size of a crowder molecule can determine the pitch of the helix.

a) Explain (graphically and with text) why crowders can change the secondary structure of a polymer, and how the pitch is determined by the crowder size (6pt).
b) What is the thermodynamic basis for helix formation (2pt)?
c) Polypeptides are one type of polymer. Why do most protein α-helices have a pitch of 0.54 nm, irrespective of the size of the crowders that surround them (2pt).

Answers

a) Draw schematic. Excluded volume around a polymer with the shape of cylindrical shell of thickness r is larger than that of the same polymer that adopts a helical configuration. The larger the crowder ratio the smaller the pitch/radius ratio.
b) The helix has a more compact structure than the cylindrical shell and thus the entropy increase and thus the free energy decreases.
c) The entropic penalty is overcome by hydrogen bonding between NH and CO groups, and these molecular interactions determine the pitch of the helix.

Q2. Vesicular transport (12 pt)

Proteins synthesized in the endoplasmic reticulum (ER) and destined for the plasma membrane (PM) travel through the secretory pathway, which involves vesicles budding off from organelles and fusing to compartments.

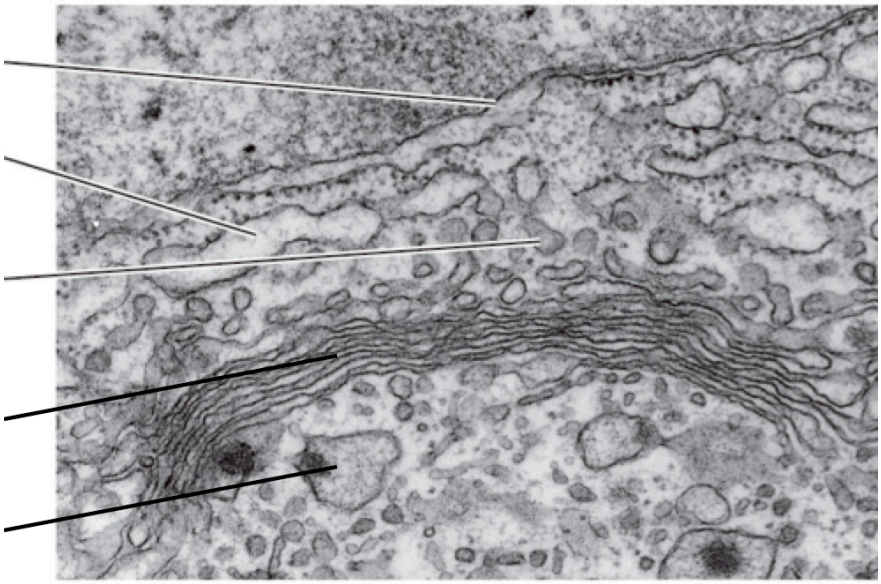
a) Describe the path of transport of a membrane protein from the ER to the PM or make scheme of the vesicle transport process. Indicate the components and describe the process of vesicles budding off from the ER and vesicle fusion in later steps (5 pt).

b) What signals the transport of a membrane protein to the PM (2pt)?

Assume the protein has arrived at the PM and metabolic conditions (availability of nutrient recognized by the membrane protein) of the cell change over time.

c) What is the fate of the membrane protein when upon a metabolic change the protein gets ubiquitinated (2pt)?

d) The image below shows part of the interior of a mammalian cell. Name the different structures that are indicated by the black lines (3pt).

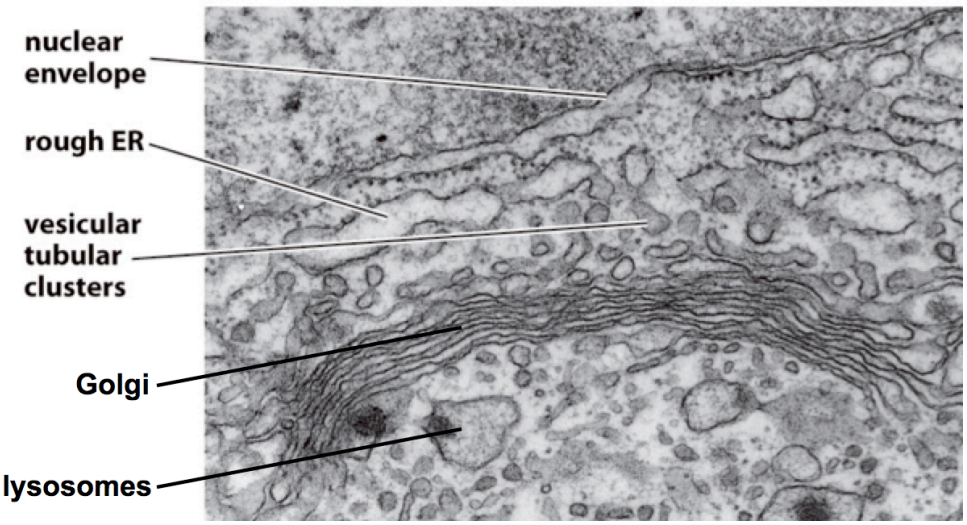


a) COPII-coating of vesicles at the ER, stimulated by nucleotide exchange factor; coat release by GTPase. Transit through Golgi network and endosomal vesicles fusing with the PM. V- and t-snare mediate fusion of the vesicles with the PM

b) No specific as the PM is the default destiniy for membrane proteins without signal. The presence of hydrophobic segments is sufficient for targeting to PM.

c) Upon ubiquitilation the protein is removed from the PM and recycled in endosomes.

d) See below.


nuclear envelope
rough ER
vesicular tubular clusters
Golgi
lysosomes

Q3. Lateral diffusion (10 pt)

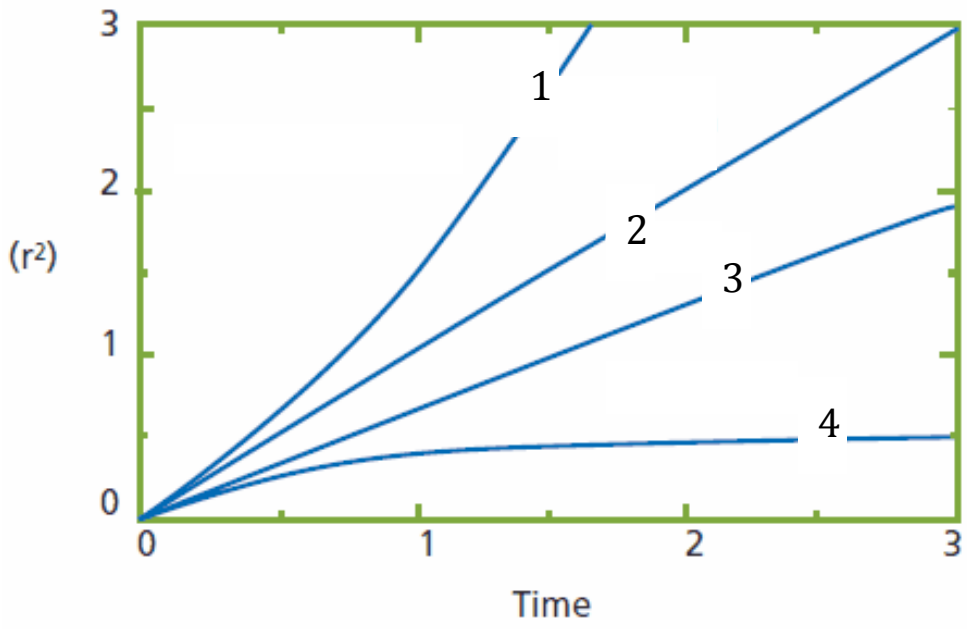
The figure below shows four scenarios of lateral diffusion of molecules in the cytoplasm of a cell. The mean square displacement (r^2) as function of time is given.

a) Describe the four regimes (1 to 4) of diffusion; what causes the different diffusion behaviours (4 pt).
b) Give the equation that describes the diffusional behaviour of curve 3 (2pt).
c) The diffusion coefficient of a 50 kDa protein in the cytoplasm of the cell is about 100 times higher than that of a similar sized protein embedded in the plasma membrane. Explain the basis for this difference and assume that the crowding is similar (2pt).
d) By averaging a few dozens of single molecule trajectories one can obtain information on the type of diffusion and accurately determine the diffusion coefficient. Nevertheless, the standard deviation is always very high. Explain (2pt).

Answers:

a) 1. Super-diffusion: active process, e.g. ATP-driven
2. Brownian diffusion, unhindered
3. Subdiffusion: due high crowding, transient interactions; diffusion in different domains in the membrane
4. Confined diffusion: molecule is confined to a specific area, where it can diffuse freely (Brownian) but cannot leave the space.

b) MSD = $r^2 = 6\,Dt^{\alpha}$; $\alpha < 1$
c) Viscosity of cytoplasm is much lower than that of the lipid membrane.
d) Movement is undirected and individual molecules travel different distances per time step but many molecules are averaged over many steps, an accurate measure of D can be obtained.



Q4. Microscopy (10 pt)

The maximum resolution of any optical system is determined by the diffraction limit. Light (wavelength, λ) travelling in a medium with refractive index *n* and converging to a spot with half-angle θ yields a spot with a radius that equals $\lambda / 2n \sin\theta$. In practice, the Abbe limit is about half the wavelength of light and thus between 200 and 300 nm for optical microscopy. In super-resolution optical microscopy one can visualize objects in the cell at a resolution of 20-50nm.

a) Explain how in super resolution microscopy objects can be seen with a resolution better than what is dictated by the Abbe diffraction limit (4pt).
b) Describe the properties of the fluorophores that are required to obtain "super-resolution" images of a cell (3pt)?
c) Design an experiment to determine by fluorescence if two different proteins (A and B) are located within their vicinity, not necessarily interacting but confined within a space smaller than 100x100 nm (3pt)

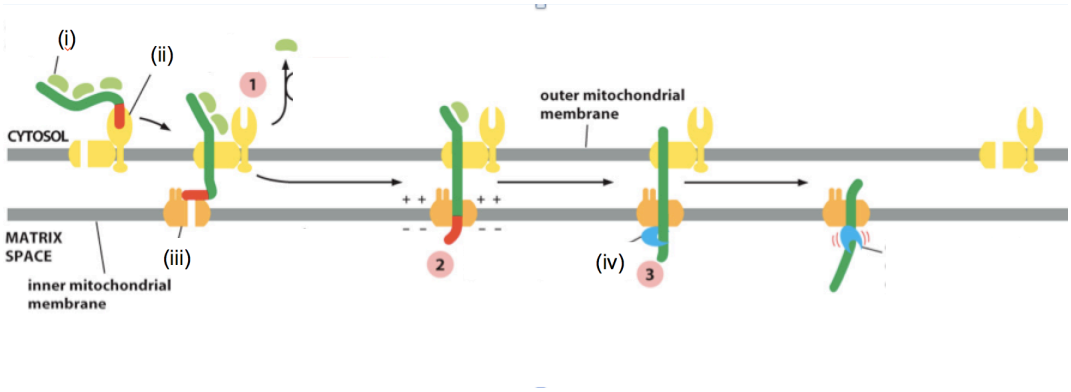
Answers:

a) In super-resolution microscopy (here PALM and STORM) the emission from two neighboring fluorescent molecules is made distinguishable by watching the fluorophores at different points of time, thus never simultaneously. Once a set of photons from a specific molecule is collected, it forms a diffraction-limited spot in the image plane of the microscope. The center of this spot can be found by fitting the observed emission profile to a known geometrical function, typically a Gaussian function in two dimensions.
b) The fluorophores need to be switchable, so that the emitted light of neighboring molecules are separated in time (and not overlapping).
c) Dual color PALM or STORM or combination of both. Label A and B with spectrally distinguishable fluorophores, e.g. green and red dye

Q5. Intracellular compartments: protein translocation (10 points)

Fully folded proteins are transported from the cytosol into the mitochondrial matrix, as shown in the scheme below.

a) Name the components (1 to 4, indicated in the scheme) that mediate the translocation of proteins into mitochondria (4pt):
   1= …
   2= …
   3= …
   4= …



b) What drives the translocation of proteins across the outer membrane (OM) and what drives the translocation across the inner membrane (IM) (3pt)?

c) How does the translocation machinery discriminate between proteins destined for mitochondrial matrix versus inner mitochondrial membrane (3pt)?

Answers:

a) See scheme
   1= cytosolic Hsp70
   2= Receptor of Tom
   3= Tim23 complex
   4= mitochondrial Hsp70

b) Across OM: cytosolic ATP; Across IM: membrane potential and ATP (via Import ATPase)

c) Stop transfer sequence in single TM proteins; internal signal sequence in the polytopic membrane proteins.

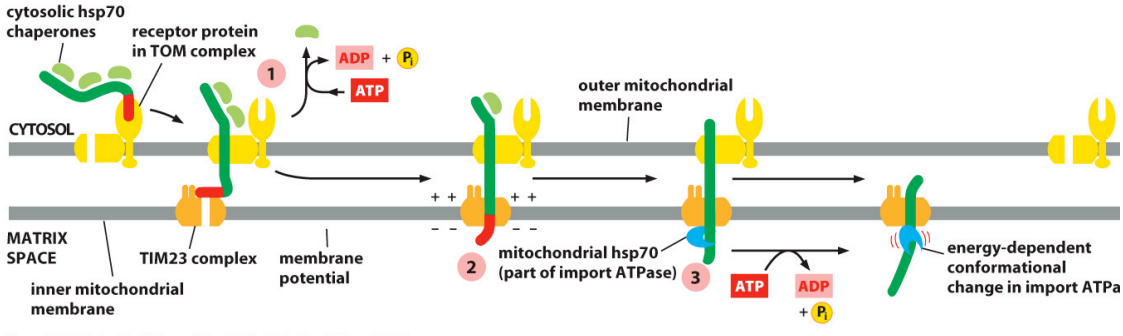


Figure 12-23 Molecular Biology of the Cell 6e (© Garland Science 2015)

Q6. Intracellular compartments: Transport in and out of the nucleus (8 points)

Fully folded proteins are transported into and out of the nucleus via the Nuclear Pore Complex (NPC). Although the pore of the NPC is much wider than the size of most molecules passing through, the transport is nevertheless selective and certain proteins are accumulated in the nucleus whereas others are excluded.

a) Make a scheme of the path of protein import into nucleus. Indicate the role of the helper proteins and indicate how selectivity is achieved (4pt)?
b) Describe the energetics of protein import into the nucleus and indicate how the transport is made uphill, that is, the NPC accumulates proteins in the nucleus (4pt).

Answers:

a) Scheme with double membrane system, pore with FG-Nups and cargo binding with NLS to karyopherins (importins) and the karyopherins interacting with the FG-Nups.
b) Transport through the NPC is disfavored by the entropy loss, which is compensated by the enthalpy gain of the karyopherins binding to the FG-Nups. The process is made uphill by the Ran-GTP gradient, which dissociates the cargo from karyopherins and release the cargo into the nucleoplasm.

Exam: Cellular Chemistry, part van Haastert (40 points)

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