Indraprastha Institute of Information Technology Delhi (IIITD)
Department of Computational Biotechnology

BIO211 – Cell Biology and Biochemistry

End-Semester Exam (December 26, 2021)

Time duration: 1.5 hours (10:00-11:30 AM) **Total marks:** 100

These answers are only for reference, the answers for students may vary a bit.
So, please read the answers carefully and award marks accordingly.

Question 1. The equilibrium constant, Keq for the following reaction ATP → ADP + P_i is $2x10^5$ M. If the measured cellular concentrations at an instance of time are [ATP] = 5mM, [ADP] = 0.5mM, and [P_i] = 5mM, is the reaction at equilibrium in living cells? **[3 marks]**

Keq = [ADP][Pi]/[ATP]
For the given concentrations [ADP][Pi]/[ATP] = (0.5mM) (5mM)/(5mM) = 0.5mM = $5x10^{-4}$M
This value is far from the equilibrium constant of the reaction ($2x10^5$M); therefore, the reaction is also far from the equilibrium. [ATP] is much higher and [ADP] is much lower that is expected at the equilibrium.

Question 2. Calculate the approximate molecular weight of a protein with 345 amino acid residues in a single polypeptide chain? **[2 marks]**
345 x 110 Da = 37950 Da

Question 3. It has been observed that proteins with diverse amino acid sequences can have similar tertiary structures. Therefore, we say protein structures are more conserved than individual amino acid sequences. Comment on this statement. **[2 marks]**
For proteins, the amino acid sequence dictates the formation of a unique folded structure. However, the reverse is not true. Many different amino acid sequences can give rise to similar folded structures. For example, the relative orientation of the charged amino acid residues in an ion pair can be switched while still preserving the overall location of the interaction. Therefore, the structures are more conserved than individual amino acid sequences.

Question 4. An enzyme has a V_{max} of 1.2 µM/s. The K_m for its substrate is 15 µM. Calculate the initial velocity, V_0 of the reaction catalyzed by this enzyme when the substrate concentration is 30 µM. **[3 marks]**
$V_0 = V_{max}$ [S] / (K_m + [S])
= (1.2 µM/s X 30 µM) / (15 µM + 30 µM)
= 0.8 µM/s

Question 5. Differentiate between **any 3** of the following: **[6 marks]**

(i) Active and passive transport

Movement of solute from a region of high concentration to a region of low concentration without additional driving force or energy is passive transportation. Whereas the movement of a solute against its concentration gradient with input of energy is termed active transport. Active transport is carried out by special types of transporters called pumps.

(ii) Paracrine and autocrine signaling

In paracrine signaling, the signal molecules diffuse locally through the extracellular fluid, remaining in the neighborhood of the cell that secretes them. Many of the signal molecules that regulate inflammation at the site of an infection or that control cell proliferation in a healing wound function in this way. In some cases, cells can respond to the local mediators that they themselves produce, a form of paracrine communication called autocrine signaling; cancer cells sometimes promote their own survival and proliferation in this way.

(iii) Heterochromatin and euchromatin

Euchromatin is an uncoiled packed and genetically active form of chromatin, while heterochromatin is a firmly packed form and is a genetically inactive part of the chromosomes.

(iv) Cohesins and condensins

The sister chromatids are held together by protein complexes called cohesins, which assemble along the length of each chromatid as the DNA is replicated. These proteins are crucial for proper chromosome segregation, and it is broken completely only in late mitosis to allow the sisters to be pulled apart by the mitotic spindle.

Condensins help in chromosome condensation when the cell enters the M phase of cell division cycle. Condensins reduce mitotic chromosomes to compact bodies that can be more easily segregated within the crowded confines of the dividing cell.

Question 6. The DNA isolated from two newly identified bacterial species (AB and XY) was found to have 14% and 38% adenine respectively.

(i) What relative proportional of adenine, guanine, thymine and cytosine are expected in these two DNA samples? What assumptions did you take into consideration for this answer? **[3 marks]**

AB: A= 14%, T=14%, G=36%, C=36%

XY: A= 38%, T=38%, G=12%, C=12%

DNA is composed of only 4 nucleotides - A, T, G and C, where A always pairs with T and G always pairs with C. Therefore, [A] = [T] and [G] = [C].

(ii) Which of these two bacterial species you think was most likely isolated from a hot water spring (82^0C), and why? **[3 marks]**

Species AB with higher G+C content is most likely the thermophilic bacteria. Its DNA will have higher melting temperature and therefore will be more stable at the temperature of the hot spring.

Question 7. While working with cultured cells, the student released that the cells can uptake glucose only when the growth medium is supplemented with Na^+, else the cells die even after having surplus glucose concentration in the culture medium? Suggest a possible explanation for this situation. **[2 marks]**

The transporter that uptakes glucose is a symport that work along with Na^+.

Question 8. Arrange the following events in order of their occurrence after a presynaptic neuron releases acetylcholine into the synaptic cleft. **[2 marks]**

(i) Vesicles carrying the neurotransmitter fuse with the cell membrane.
(ii) Ligand-gated Na^+ channels open, causing an influx of Na^+ ions.
(iii) Voltage-gated Na^+ channels open in the axon.
(iv) Membrane depolarization triggers voltage-gated Ca^{2+} channels to open.
(v) Local membrane depolarization in the axon triggers an efflux of K^+.

ii, iii, v, iv, i

Question 9. The actual free energy of hydrolysis of ATP under intracellular conditions (ΔG) differs from the standard free-energy change (ΔG'°).

(i) Calculate ΔG for the hydrolysis of ATP in human erythrocytes, given that the standard free energy of hydrolysis of ATP is -30.5 kJ/mol and the concentrations of ATP, ADP, and P_i are 2.25, 0.25, and 1.65 mM, respectively. Assume pH to be 7.0, temperature to be 37°C and gas constant, R to be 8.315 J/mol.K. **[5 marks]**

$$\Delta G_p = \Delta G'^\circ + RT \ln \frac{[ADP][P_i]}{[ATP]}$$

Substituting the appropriate values we obtain

$$\Delta G_p = -30.5 \text{ kJ/mol} + \left[(8.315 \text{ J/mol} \cdot \text{K})(298 \text{ K}) \ln \frac{(0.25 \times 10^{-3})(1.65 \times 10^{-3})}{2.25 \times 10^{-3}}\right]$$

$$= -30.5 \text{ kJ/mol} + (2.48 \text{ kJ/mol}) \ln 1.8 \times 10^{-4}$$
$$= -30.5 \text{ kJ/mol} - 21 \text{ kJ/mol}$$
$$= -52 \text{ kJ/mol}$$

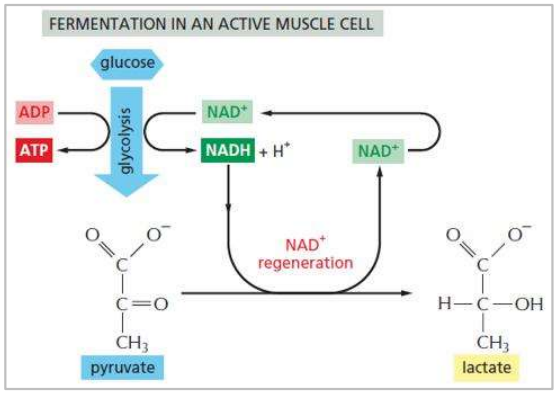
(ii) What can you say about the amount of energy required to synthesize ATP under the same cellular conditions? **[2 marks]**

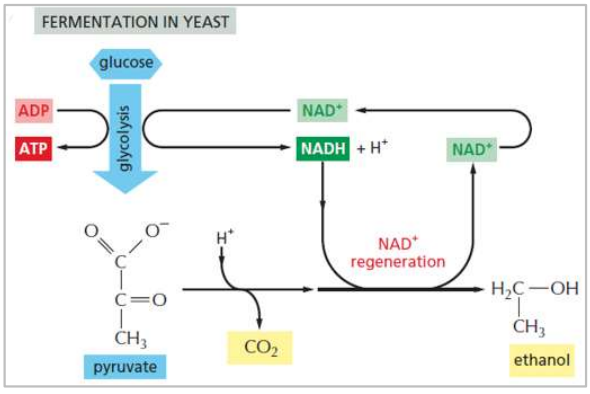
The free energy required to synthesize ATP from ADP and Pi under the conditions prevailing in the erythrocyte would be 52 kJ/mol.

Question 10. Briefly discuss the three catabolic routes through which pyruvate formed by glycolysis is further metabolized. **[6 marks]**

Pyruvate formed by glycolysis is further metabolized via one of three catabolic routes:

1. In aerobic organisms or tissues, under aerobic conditions, glycolysis is only the first stage in the complete degradation of glucose. Pyruvate is oxidized, with loss of its carboxyl group as CO_2, to yield the acetyl group of acetyl-coenzyme A; the acetyl group is then oxidized completely to CO_2 by the citric acid cycle. The electrons from these oxidations are passed to O_2 through a chain of carriers in the mitochondrion, to form H_2O. The energy from the electron-transfer reactions drives the synthesis of ATP in the mitochondrion.

2. 

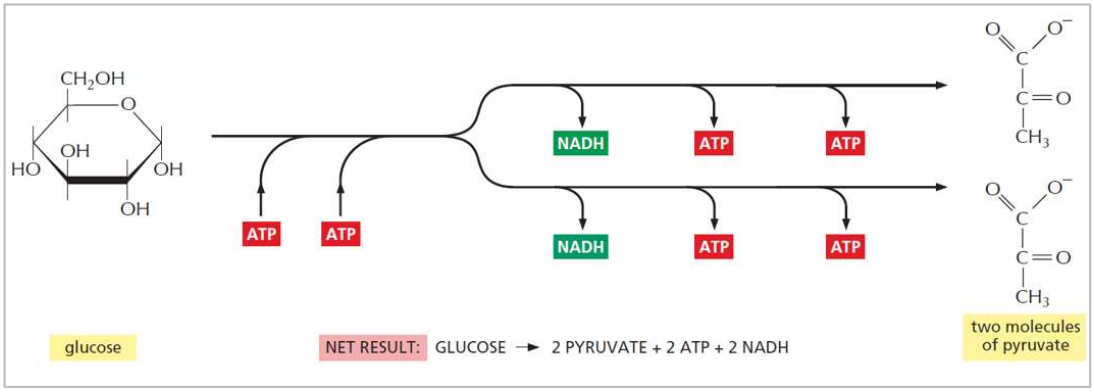
3. 

OR

Answer the following:

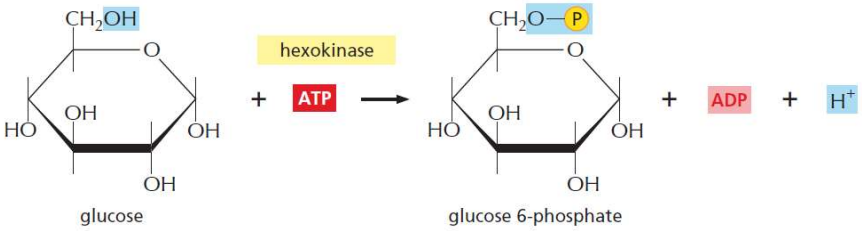
(i) Why phosphorylation of glycolytic intermediates considered important?
To retain phosphorylated intermediates in the cell.

(ii) Give the net equation of the overall glycolytic process.

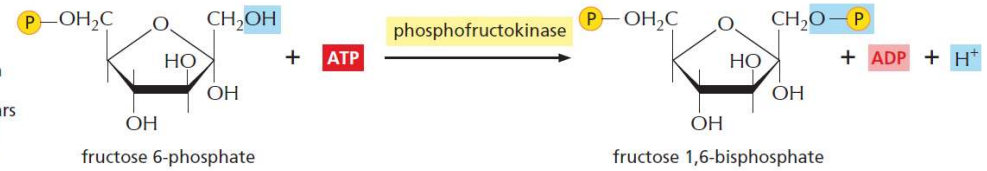


(iii) Give the three steps in glycolysis that strongly favor the direction of glucose breakdown making the process effectively irreversible.

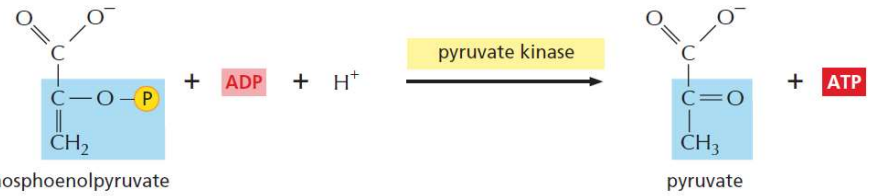
Step 1 Glucose is phosphorylated by ATP to form a sugar phosphate. The negative charge of the phosphate prevents passage of the sugar phosphate through the plasma membrane, trapping glucose inside the cell.



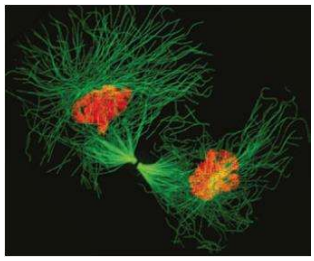
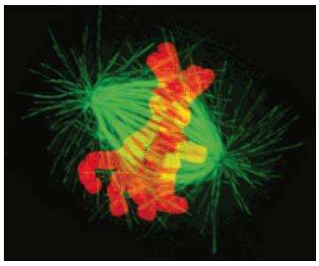
Step 3 The new hydroxyl group on carbon 1 is phosphorylated by ATP, in preparation for the formation of two three-carbon sugar phosphates. The entry of sugars into glycolysis is controlled at this step, through regulation of the enzyme *phosphofructokinase*.



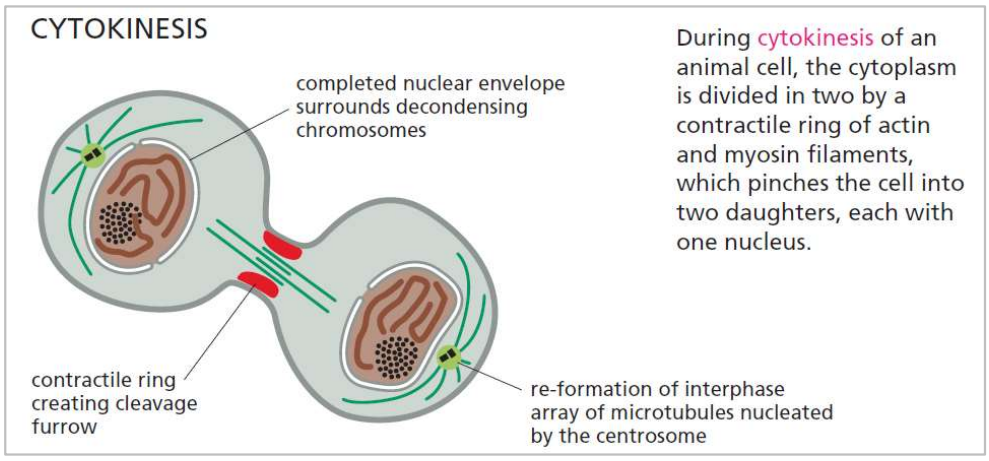
Step 10 The transfer to ADP of the high-energy phosphate group that was generated in step 9 forms ATP, completing glycolysis.



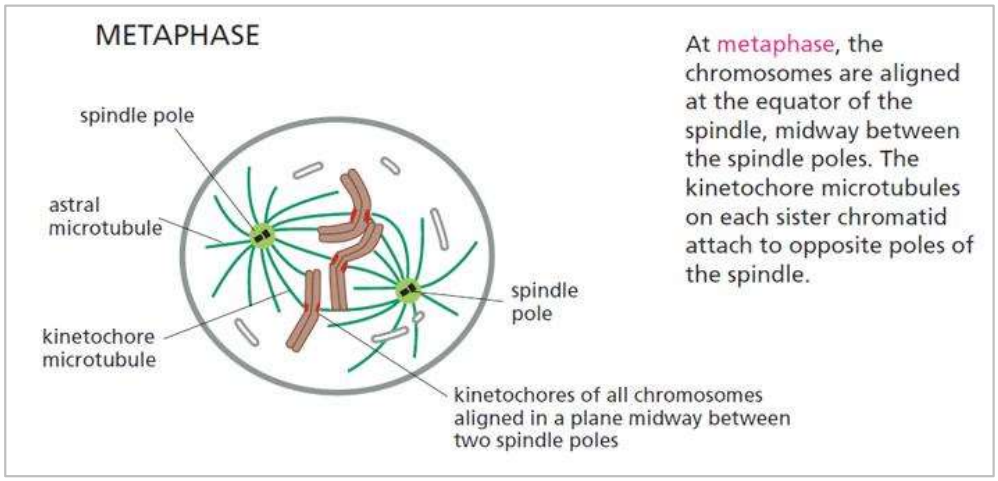
<u>Question 11.</u> Identify the stage of cell division process shown in the given figures. List the characteristic features observed during these stages. **[2 x 5 marks]**

A.  B. 

A. Cytokinesis



B. Metaphase



Question 12. State whether the given statement is TRUE or FALSE. **[10 marks]**

(i) The hereditary information of a cell is passed on by its proteins. False

(ii) Bacterial DNA is enclosed in a nucleus located in the cytosol. False

(iii) Hydrogen bonds are weak and can be broken by thermal energy, yet they contribute significantly to the specificity of interactions between macromolecules. True

(iv) The initiator tRNA enters the ribosome from E site. False

(v) Linking the reaction X → Y to a second, energetically favorable reaction Y → Z will shift the equilibrium constant of the first reaction. False

(vi) Okazaki fragments are removed by a nuclease that degrades RNA. False

(vii) Amino acids and lipids are amphipathic in nature. False

(viii) Lipids in a lipid bilayer do not flip-flop readily from one lipid monolayer to the other. True

(ix) Ubiquinone and cytochrome c are both diffusible electron carriers. True

(x) The enzymatic activity of a Cdk protein is determined both by the presence of a bound cyclin and by the phosphorylation state of the Cdk. True

Question 13. What are the different components of replication machinery? Explain the function of each of these components. **[6 marks]**

DNA helicase sits at the very front of the replication machine where it uses the energy of ATP hydrolysis to propel itself forward, pulling apart the double helix as it speeds along the DNA.

Single strand DNA-binding proteins cling to the single-stranded DNA exposed by the helicase, transiently preventing the strands from re-forming base pairs and keeping them in an elongated form so that they can serve as efficient templates.

DNA polymerase catalyzes the addition of nucleotides to the 3′ end of a growing DNA strand, using one of the original, parental DNA strands as a template.

Sliding clamp keeps DNA polymerase firmly attached to the template while it is synthesizing new strands of DNA. Clamp loader, which hydrolyzes ATP each time it locks a sliding clamp around a newly formed DNA double helix.

RNA polymerase called primase helps in synthesizing the primer providing a base-paired 3′ end as a starting point.

To produce a continuous new DNA strand from the many separate pieces of nucleic acid made on the lagging strand, three additional enzymes are needed.

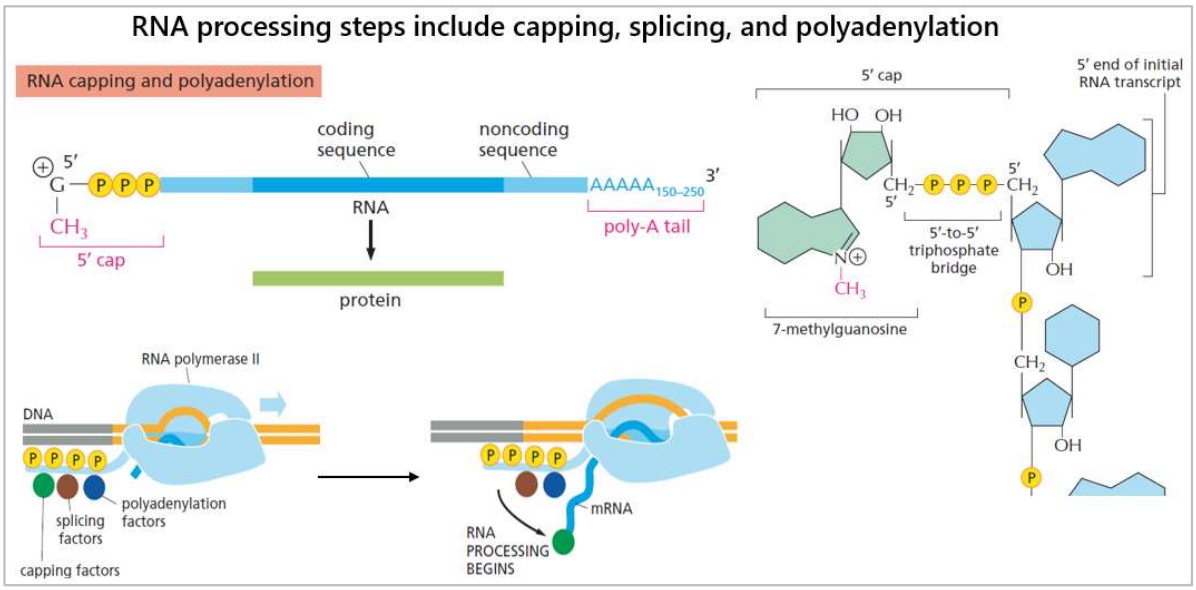
Nuclease degrades the RNA primer.

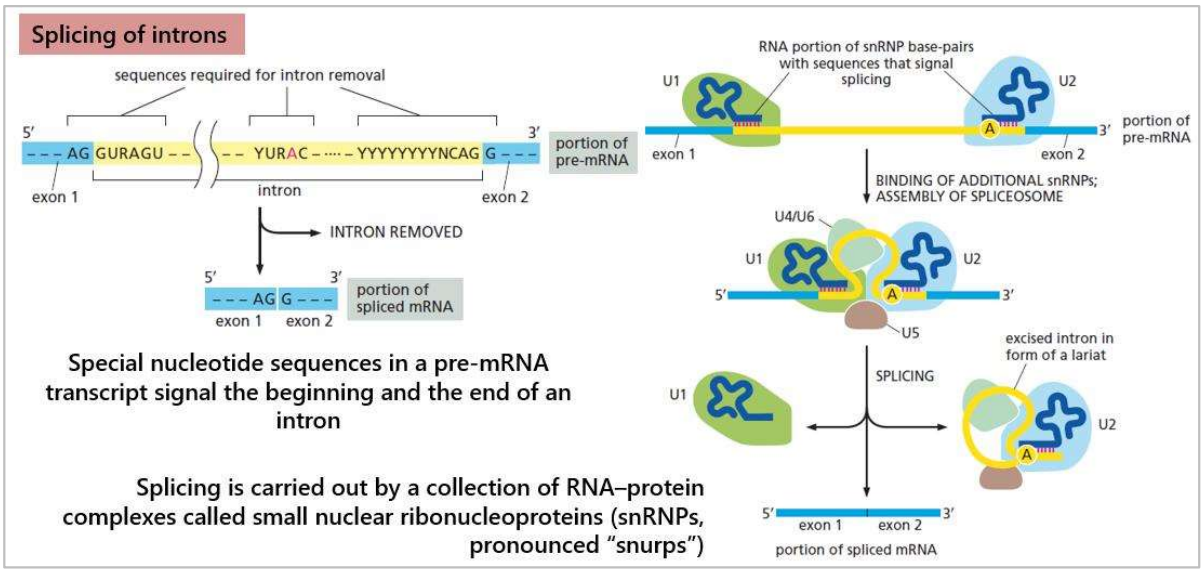
DNA polymerase called a repair polymerase replaces this RNA with DNA (using the end of the adjacent Okazaki fragment as a primer).

DNA ligase joins the 5′-phosphate end of one DNA fragment to the adjacent 3′-hydroxyl end of the next.

OR

Briefly describe the processing steps required to convert precursor mRNA to a matured mRNA. Also mention the cellular location for each of these processing steps.





All these processes take place in the nucleus.

Question 14. Show the rearrangement of Michaelis-Menten equation for Lineweaver-Burk representation. Draw a schematic of Lineweaver-Burk plot and mark the various parameters related to enzyme kinetics that can be estimated from the plot. **[4 marks]**

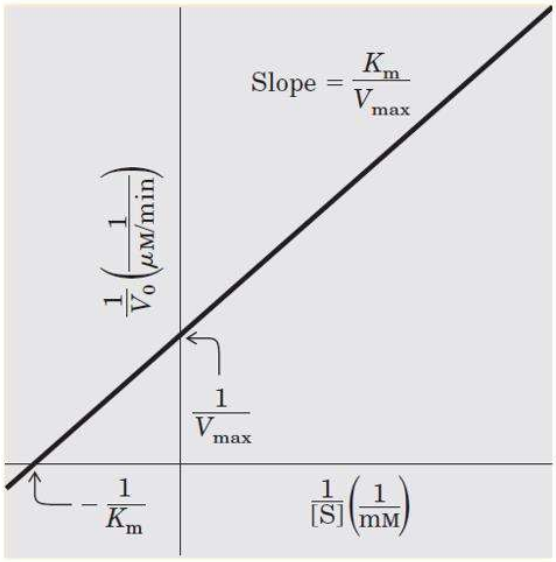
Rewritten in the following manner, the equation gives a straight line, which is useful to determinate the parameters K_m and V_{max} (Lineweaver-Burk representation):

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{1}{V_0} = \frac{K_m + [S]}{V_{max}[S]}$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

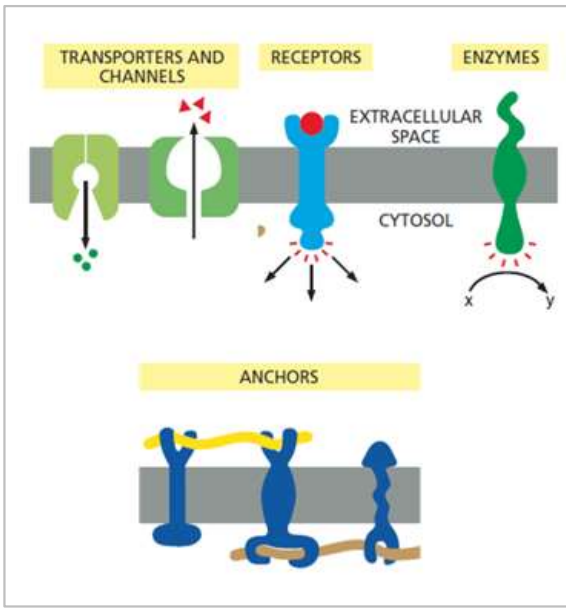


Question 15. Explain the two factors that determine the fluidity of the lipid bilayer membrane? **[4 marks]**

Fluidity a lipid bilayer at a given temperature depends on its phospholipid composition and on the nature of the hydrocarbon tails: the closer and more regular the packing of the tails, the more viscous and less fluid the bilayer will be.

Two major properties of hydrocarbon tails affect how tightly they pack in the bilayer: their length and the number of double bonds they contain. A shorter chain length reduces the tendency of the hydrocarbon tails to interact with one another and therefore increases the fluidity of the bilayer. The hydrocarbon tails of membrane phospholipids vary in length between 14 and 24 carbon atoms, with 18–20 atoms being most usual. Most phospholipids contain one hydrocarbon tail that has one or more double bonds between adjacent carbon atoms, and a second tail with single bonds only. Each double bond in an unsaturated tail creates a small kink in the tail, which makes it more difficult for the tails to pack against one another.

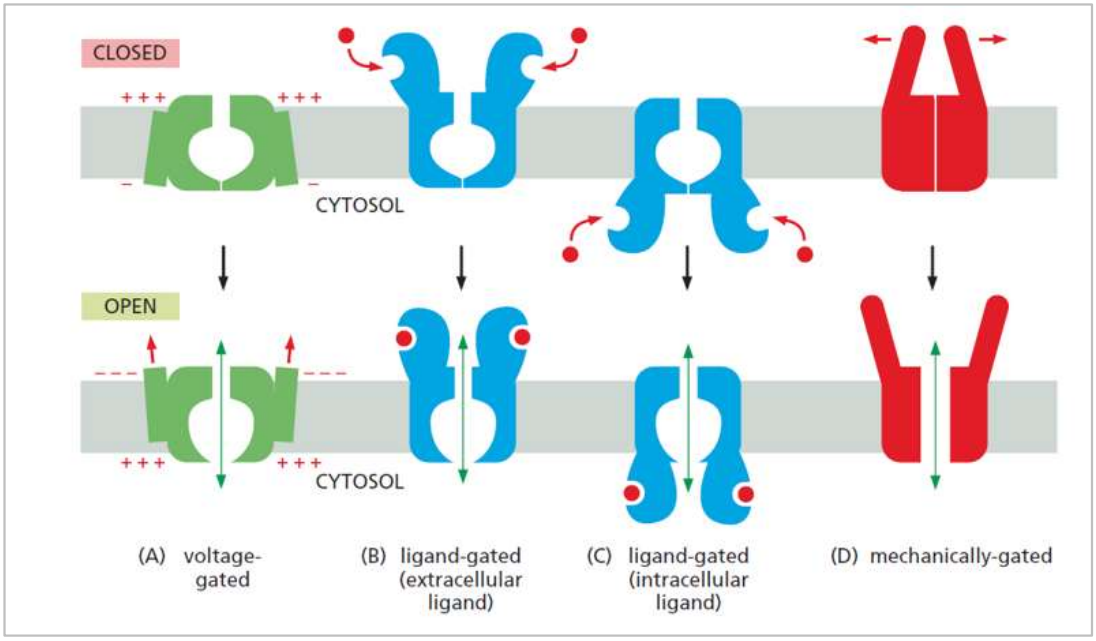
Question 16. What are the different functional classes of membrane proteins? Give an example of each. **[5 marks]**



Functional Class	Protein Example
Transporters	Na^+ pump
Ion channels	K^+ leak channel
Anchors	integrins
Receptors	platelet-derived growth factor (PDGF) receptor
Enzymes	adenylyl cyclase

OR

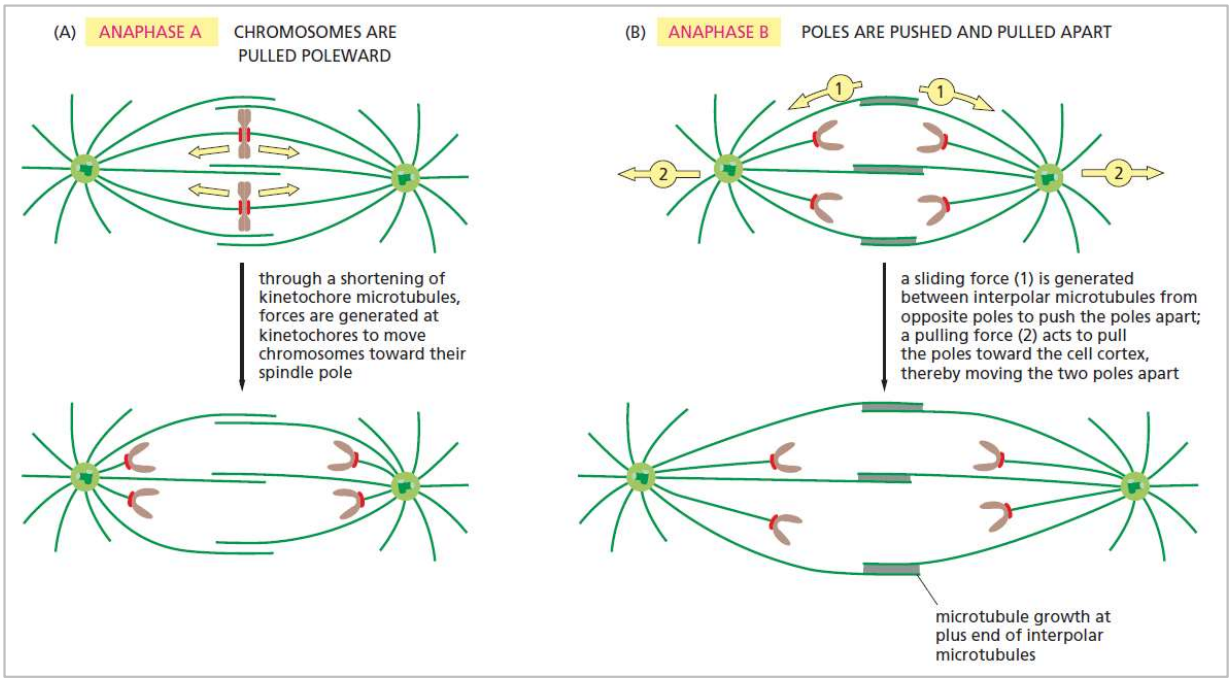
What are the three ways in which an ion channel can be gated? Explain with the help of a diagram.



Question 17. Arrange the following in increasing rate of simple diffusion across the lipid bilayer membrane: Glucose, CO_2, Na^+, Water **[2 marks]**

$Na^+ < Glucose < Water < CO_2$

Question 18. Explain the two processes that segregate daughter chromosomes at anaphase. **[5 marks]**



Question 19. Give an example in each of the following categories of extracellular signaling molecules: **[2 marks]**

(i) Hormone
(ii) Local mediator
(iii) Neurotransmitter
(iv) Contact-dependent signaling molecule

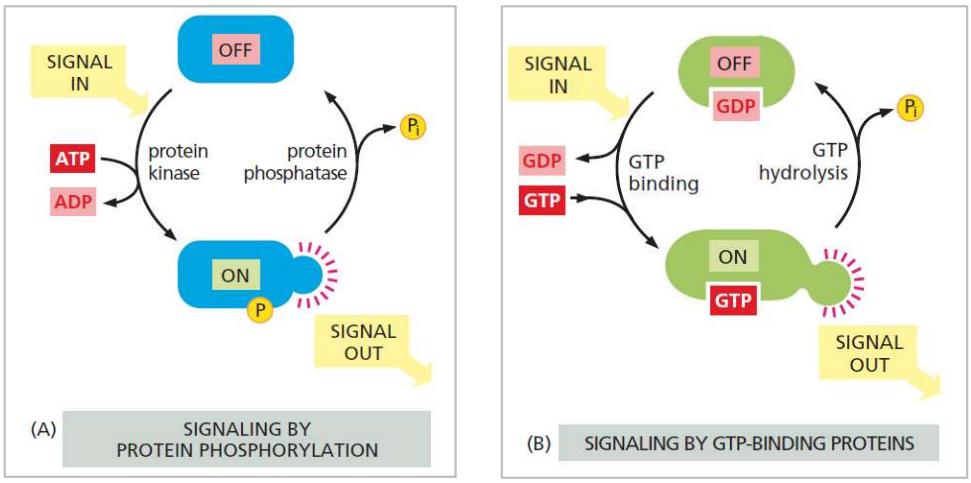
Hormones
Adrenaline (epinephrine)
Cortisol
Estradiol
Insulin
Testosterone
Thyroid hormone (thyroxine)

Local Mediators
Epidermal growth factor (EGF)
Platelet-derived growth factor (PDGF)
Nerve growth factor (NGF)
Histamine
Nitric oxide (NO)

Neurotransmitters
Acetylcholine
γ-Aminobutyric acid (GABA)

Contact-dependent Signal
Delta

Question 20. Describe the two major classes of intracellular signaling proteins that act as molecular switches. **[4 marks]**



Question 21. Match the following foreign substances to the normal cellular signal they relate to. **[3 marks]**

A. Barbiturates	i. Cold
B. Morphine	ii. Acetylcholine
C. Menthol	iii. Glycine
D. Capsaicin	iv. Endorphin
E. Nicotine	v. Gamma-aminobutyric acid
F. Strychnine	vi. Heat

A-v, B-iv, C-i, D-vi, E-ii, F-iii

Question 22. What are the three main transition points in the cell-cycle that are regulated by the cell-cycle control system? Mention one mechanism used by the control system to pause the cycle at each of these specific transition points. **[6 marks]**