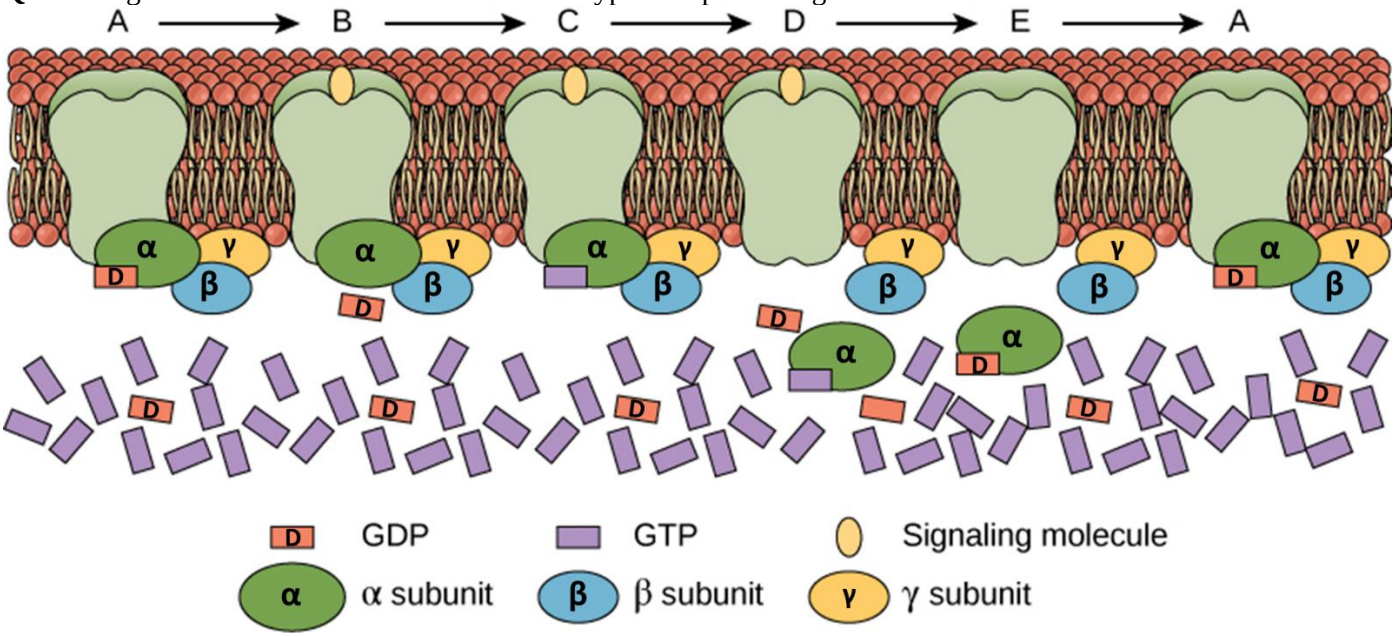
Birla Institute of Technology & Science (BITS), Pilani
Intro to Mol Bio and Immuno (PHA F215); **Second Semester 2022-23**
Comprehensive Exam (**Closed Book**), 11th May 2023; **Maximum Marks: 40**, **Time: 90 Min**

Q1. The figure below shows a series of states for typical G protein signal transduction.



Use this representation to describe the following stages in this signaling process:

i. between A and B
ii. between B and C
iii. between C and D
iv. between D and E
v. between E and A **[3]**

Q2. a) What types of signaling molecules (chemical nature) typically use cell surface receptors to initiate response events in cells? **b)** Describe in general terms the role of protein kinases in cellular signaling. **c)** Most phosphoprotein phosphatases in eukaryotic cells are constitutively active. Why is this important to the overall process of cell signaling? **[2+2+2]**

Q3. The Fc regions of antibodies come in many flavors and have many different functions. List three different Fc regions and explain their functional roles in the immune system. **[5]**

Q4. There are many types of 7-transmembrane receptors, including epinephrine, acetylcholine, and serotonin receptor, which binds exclusively to their cognate ligands. Though they have different receptors, binding of the ligand to their respective receptors leads to similar cellular activity. Explain how it is possible that two different signaling molecules binding to their respective seven-pass receptors can bring about similar responses within a cell. **[3]**

Q5. Explain how antigen presenting cells control both humoral and cell-mediated immunity. **[3]**

Q6. a) What is the importance of having both extra and intra-cellular TLR receptors? **b)** What are the similarities between the function of complements and antibodies? **c)** Why do we need antibodies when complements can do most of their function? **[2+3+3]**

Q7. What is the importance of both B-cell epitope and T-cell epitope in an antigen? If a protein antigen lacks a T-cell epitope but has a B-cell epitope, what will be the consequence? **[3]**

Q8. Why tetanus toxoid is primarily used for making conjugate vaccines? What are the prerequisites for selecting a suitable vector for DNA (carrier) vaccines? **[3+3]**

Q9. Write a note on germinal center reaction and affinity maturation. **[3]**

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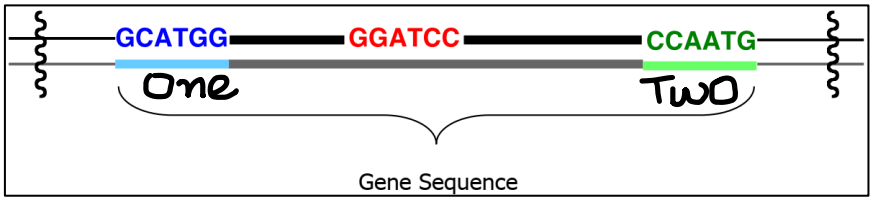
Q1. The Polymerase Chain Reaction (PCR) is a useful tool to amplify specific DNA sequences. A gene sequence within a long stretch of DNA is shown below diagrammatically. The upper strand is the coding strand, and the lower strand is the template strand.



a) Write the DNA base sequences corresponding to the "One" and the "Two" regions on the bottom strand. Be sure to designate 5' and 3' ends of your sequences. **[2]**

b) Write the sequences of the two primers needed to amplify the gene beginning with the blue sequence and ending with the green sequence. Be sure to designate 5' and 3' ends. **[2]**

c) Draw the hybridized DNA that would form if you took only the top strand of DNA and mixed it with the two primers from part **(b)**. **[2]**

d) After 10 cycles of PCR using the two primers from part (b), how many total strands of DNA will you have? **[2]**

e) Draw the possible resulting products after 1 round of PCR if, in addition to the two primers used in part **(b)**, a third primer with a sequence identical to the middle region (-GGATCC-) was added. **[3]**

Q2. Write the **best** answers in the spaces below (one word only):

To divide properly, a cell must first copy its entire genome. _____is the enzyme responsible for elongating DNA daughter strands during DNA _____. The extension of daughter strands always proceeds by the addition of nucleotides to the _____-prime end of the growing strand.

On a chromosome, DNA replication takes place at localized regions referred to as replication _____, which move along the DNA double helix. One strand, called the_____ strand, is synthesized continuously while the other strand, called the _____strand, is synthesized initially as a series of so-called _____ fragments. When errors are introduced during daughter strand elongation, DNA polymerase can remove miss-incorporated bases using it's 3' to 5' _____ activity. **[4]**

Q3. The structure of the enzyme tryptophan synthetase has been studied extensively by a variety of methods. In a series of studies, Yanofsky and coworkers examined the effect on enzyme activity of various amino acid changes in the protein sequence. Altered amino acids are shown in bold. "Wild-type" is the normal strain isolated from the wild.

Strain	Amino Acid at Position A	Enzymatic Activity
wild-type	Gly	Full
mutant 1	Glu	none
mutant 2	Arg	none

Here are two possible explanations for these results:

- The Gly ⇒ Glu and Gly ⇒ Arg changes introduce a charge (+) or (–) into a region of the protein that requires an uncharged amino acid like glycine.
- The Gly ⇒ Glu and Gly ⇒ Arg changes introduce much larger amino acid side chains into a space in the protein that requires a small amino acid like glycine.

Yanofsky and coworkers collected more mutants and examined their proteins to determine which of the above explanations was more likely to be correct:

Strain	Amino Acid at Position A	Enzymatic Activity
wild-type	Gly	full
mutant 3	Ser	full
mutant 4	Ala	full
mutant 5	Val	partial

Which of their models is supported by these data? Why? **[4]**

PTO

Q4. Assume that Protein A is a 5 amino acid protein, the sequence of which is indicated below. Each of these amino acids is critical for the proper folding of this protein: N - pro-asn-ser-met-leu-C.
The DNA sequence encoding the above 5 amino acids is included within the sequence below.

5′-AACCGAATTCCATGTTATAGC-3'
3′-TTGGCTTAAGGTACAATATCG-5′

You have been given the following two different mutant alleles of Gene A. Each mutant allele is due to a point mutation that is bold and underlined. Which of these mutants will ALTER the folding of Protein A? Explain. **[3]**

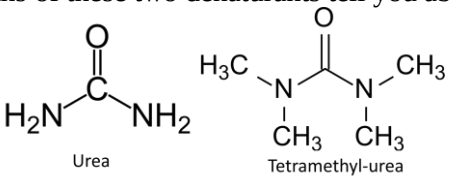
Mutant 1

5′-AACC**A**AATTCCATGTTATAGC-3′
3′-TTGG**T**TTAAGGTACAATATCG-5′

Mutant 2

5′-AACCG**T**ATTCCATGTTATAGC-3′
3′-TTGGC**A**TAAGGTACAATATCG-5′

Q5. It is thought that urea denatures DNA by disrupting H-bonds between the base pairs. However, it was found that tetramethylurea is a much better denaturant than the unsubstituted urea. Based on their differences in structure, what does the relative difference in the strengths of these two denaturants tell you about how urea denatures DNA? **[3]**



Q6. The melatonin receptor exists in different isoforms: MT1, encoded by the MT-1 gene, and MT2, encoded by the MT-2 gene. The following sequence corresponds to 90-140 base pairs (bp) of MT-1 and MT- 2 genes.

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                90                                                  140
MT-1: 5′------GATATGCCCCCCCCGGCGCGCGATATGCCCCCCCCGGCGCGCGTGCGTGA-------3′
      3′------CTATACGGGGGGGGCCGCGCGCTATACGGGGGGGGCCGCGCGCACGCACT-------5′

                90                                                  140
MT-2: 5′------GATATGATATATATATATATAGATATGAAAAATTTTTATATAGTGCGTGA-------3′
      3′------CTATACTATATATATATATATCTATACTTTTTAAAAAATTATCACGCACT-------5′
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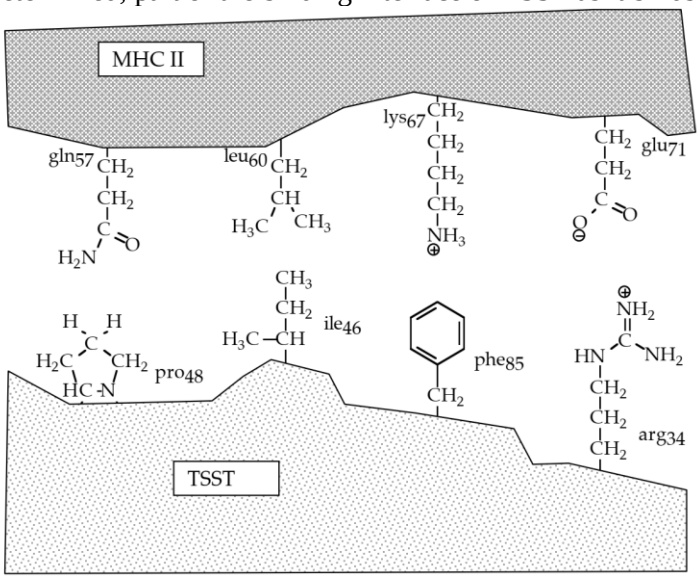
a) Which of the above sequence will denature at a lower temperature and why? **[3]**
b) If you denature a DNA duplex and a protein, which macromolecule is likely to renature and why? **[3]**

Q5. Toxic Shock Syndrome Toxin (TSST) is a protein produced by the bacterium *Staphylococcus aureus*. During an *S. aureus* infection, the TSST protein binds to MHC Class II proteins (MHC II) found on the surface of antigen-presenting cells of the patient's immune system. The binding of TSST to MHC II results in hyperactivation of the immune cells, which leads to the symptoms of toxic shock syndrome. A simplified version of the structure of both proteins has been determined; part of the binding interface of TSST as it binds to MHC II is shown below. **[3×3]**



a) Suppose you wanted to design an altered version of either MHC II or TSST that would make the interaction between TSST and MHC II stronger than in the normal situation. Which amino acid would you change and what would you change it to?

b) You prepared few altered versions of the TSST protein. Version 1 of TSST ($TSST_1$; normal TSST is called $TSST_{Norm}$) has a glutamine at position 34 instead of an arginine. Under conditions where $TSST_{Norm}$ would bind to MHC II, $TSST_1$ does not bind. Provide a reasonable explanation for why $TSST_1$ does not bind.

c) Version 2 of TSST ($TSST_2$) has a leucine at position 46 instead of an isoleucine. Under conditions where $TSST_{Norm}$ would bind to MHC II, $TSST_2$ does bind. Provide a reasonable explanation for why $TSST_2$ does bind.

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