Instructions
(Prelims Round)

30th June, 2021

Read the following instructions carefully before attempting the question paper:

- The exam starts at **14:00:00 IST, 30th June 2021**. The duration of the exam is 10 hours inclusive of time taken for any unforeseen delays in scanning and uploading. The hard deadline is set at **23:59:59 IST, 30th June 2021**. Any submission after that will not be considered. **Only one** submission per team will be allowed.
- We highly encourage you to start the process of submitting your answers in the Google Form (see below) by 23:00 hours IST. Late submissions, in any case, will not be accepted. If you face persisting network issues during submission, inform us immediately by email (bioblitz.pravega@gmail.com).
- This paper contains **6 questions** in total. All questions are **compulsory**, so

answer all of them to the best of your abilities. The first question involves 12 subparts which intend to touch upon diverse facets of biological sciences. There are **31 pages** in this question paper. A table of the standard genetic code has been provided in the **last page** of this question booklet.

- Answers can be either handwritten or typed.
- Mention question number and title (which is given at the beginning of each question) clearly. It is advisable to write the question number at the middle of the page to avoid it being cut out during scanning. Answer all subparts of a question together and begin each question in new page. Clearly mention the subpart number for each question.
- Clearly write your name(s), the name of your institute, as well as your registered email ID's at the beginning of the answer script.
- Submit **only one** PDF document. Do **not** submit multiple documents. Any scanned images, diagrams, or types/handwritten answers must be compiled in the same document. The file name **must** follow the format: *Participant#1FirstName_ Participant#2FirstName_ Participant#3FirstName.pdf*. Please make **absolutely** sure you upload the correct document and that all pages are included as you expect them to be, as you shall **not** be able to change this once it has been submitted.
- Show your steps **clearly** for all the questions (within the word/sentence limit, if applicable, of course). Do not skip steps to receive full credit. Partial credit may be awarded for an incomplete solution or progress towards a solution.
- There is **no negative marking**, but while selection, in case of a tie, those with lesser incorrect answers will be given preference.
- The coordinators of the event shall be available in two separate slots during the course of the examination so that you can clarify any doubts that come to your mind while writing the exam. It is **highly encouraged** that you communicate your doubt during these **clarification sessions** instead of wasting your time in writing long emails to the organizers in order to explain your point. The slots chosen are **15:00-15:30 IST and 19:00-19:30 IST** on the day of the Prelims (i.e. 30$^{\text{th}}$ June, 2021). The link for both the calls to be organized on **Google Meet** is provided herewith: https://meet.google.com/coc-vxhi-xuy.
- In the event that any further doubts persist, kindly **mail** the organizers at the email ID mentioned previously. The organizers will try their best to clarify. Even after that, if you feel you have any comments regarding the question (for example, incompleteness, incorrectness, etc.) you can mention that in your answer clearly with **all** the reasons why you think so and/or with the alternate assumption you took to arrive at your answer. If your reasoning is correct, scoring will be done accordingly. But the first priority should be getting it clarified from us.

- You are free to do beyond what is asked to do in the question (though it will not be considered for evaluation). You are also free to add any comments in your answer regarding any question.
- The exam is an **open-book** one. You may consult any non-living sources such as books, internet etc but you must **properly cite** the sources if you use them. In case you fail to do so, you will be penalised on grounds of plagiarism, and it may even lead to disqualification of the team.
- Indicate clearly the presence of all material you have quoted from other sources, including any diagrams, charts, tables, or graphs. Even while taking help of some inanimate resource, avoid direct quotes and paraphrase adequately all material in your own words.
- While using the internet may be tempting, do keep in mind that the time is limited and you derive the greatest benefit from using your own thinking faculties.
- You must ensure that the submitted work is entirely your own and you have not used the services of any agency or person(s) providing specimen, model or ghostwritten work in the preparation of the work you submit for this open-book test. You must not give assistance in providing the same to other candidates submitting for this open-book test as well.
- If there are any corrections to any question, those will be informed to you over mail.

Submissions **MUST** be made using the form:
https://bit.ly/BioBlitzPrelimsSubmission

> *"The time ... has arrived when biology must, like the other sciences, make a fresh start in a purely speculative direction..."*

This exam is not an entrance examination, nor is it determinant of your college grades. The question setters have put in a great deal of effort in framing the questions, and would expect your sincerity and integrity in answering the same. Some questions are difficult, however, the winner shall be the one who can snake through the entire paper without squandering time on the more difficult ones. Above all, this is a fun-filled biological extravaganza, so enjoy solving the questions. Hope this is a great learning experience for you.

Best of Luck!

Questions
(225 points)

1 (*74 points*) The 'Blitz'krieg (Short Answer Type)

1.1

A harmless X-linked recessive mutation, let's call it A, affects only 1% of the population. What percentage of the population has the X^A chromosome?

(*2 points*)

1.2

In an experiment, a plant cell was placed in a solution containing 1 mM of Ca^{2+} ions. The electrochemical concentration across the membrane was found to be -110 mV. Nernst equation predicted that the intracellular concentration of calcium ions should be 5400 mM. The actual concentration was found to be 1.5 mM. What can you infer about the mode of transport of Ca^{2+} from this set of information? Justify your answer in **not more than two sentences**.

(*3 points*)

1.3

1.3.1

The Coefficient of Relatedness (r) is defined as the degree of genetic similarity between two organisms. For example, the value of r for you and your father is 0.5, because you share 50% of your genes with him.

(a) Calculate the value of r for two full siblings. Does it make a difference of the siblings are of different genders? Explain in 1-2 lines.

(*1 + 2 = 3 points*)

(b) Is it possible to calculate the coefficient of relatedness accurately for the following to cases? Justify your response in 1 line in each of the cases.

(I) A naturally occurring population.

(II) Two fruit flies obtained from a typical dihybrid cross in a lab.

(III) Two cancerous cells (2^{nd} generation) present that are derived from the same parent cancerous cell (0^{th} generation).

(1 + 1 + 1 = 3 points)

1.3.2

Simply stated, Hamilton's rule says that altruistic cooperation is favoured if the benefits (B) to the recipient (the organism who receives the altruistic 'help'), weighted by the coefficient of relatedness (r) of the recipient to the actor (who performs the altruistic deed), outweigh the costs (C) to the actor. Mathematically, altruism is favored if $rB > C$.
Why do you think the coefficient of relatedness is important here? Why can't only the cost and benefit be considered when the other individual is a relative?

(2 + 2 = 4 points)

1.3.3

Arizonan tiger salamanders demonstrate cannibalism. Tiger salamander larvae occur in two morphs, a 'typical' or 'normal' morph that feeds on invertebrate prey, and a larger physically distinctive 'cannibal' morph that has specialized oral structures to facilitate said cannibalism.

(a) Propose and justify two possible scenarios where cannibalism is helpful/essential for survival of individuals.

(2 + 2 = 4 points)

(b) Do you think that altruism would play a role in cannibalism? How will that reflect in cannibalistic

behavior? Can you propose an experiment to test this?

(1 + 2 + 2 = 5 points)

(c) Scientists carrying out experiments found two kinds of cannibals: discriminators and non-discriminators (based on kin discrimination). Draw a relevant graph/plot that shows your results if the previously proposed experiment is carried out for both these types of cannibals.

(2 + 2 = 4 points)

1.4

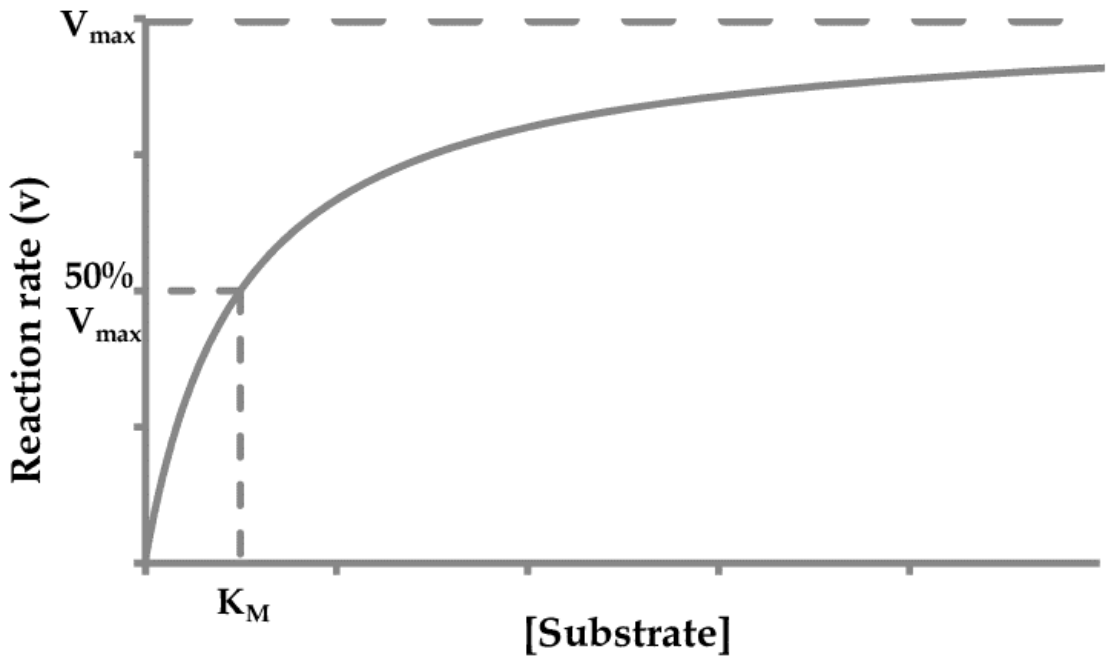
Achintya receives radiation therapy for a squamous cell carcinoma of the lung. Despite therapy, the tumour progressively increases in size, and he dies 6 months later. His tumour cells contain a point mutation in the p53 gene, leading to an inactive gene product. Based on this finding, the progressive tumour growth

despite irradiation therapy is most likely to be related to a defect in cell cycle arrest in which of the following phases of the cell cycle?

(*2 points*)

1.5

Consider the Michaelis-Menten model of enzyme kinetics and answer the questions that follow:



(a) State whether the following statements are true or false:

(i) At saturating levels of substrate, the rate of an enzyme catalysed reaction is proportional to the enzyme concentration.

(ii) At saturating levels of substrate, the rate of an enzyme catalysed reaction is proportional to the substrate concentration.

(iii) The rate of an enzyme catalysed reaction in the presence of a rate-limiting concentration of substrate decreases with time.

(iv) The affinity of an allosteric enzyme for substrate varies with enzyme concentration.

(*1 × 4 = 4 points*)

(b) Two enzymes A and B have Michaelis constants of 9.5×10^{-5} and 4.4×10^{-1} respectively.

(I) Which one has greater catalytic efficiency?

(II) Which one has greater substrate affinity?

Justify your answers in **not more than one sentence** in each case.

(2 + 2 = 4 points)

1.6

The mutation A causes the protein the gene codes for, to split into two. Given it is a transversion in the wobble base of a non-aromatic amino acid, resulting in a nonsense mutation, identify the amino acid and in **not more than 50 words**, explain why did the protein split.

(1.5 + 2.5 = 4 points)

1.7

50 years from now, a synthetic biologist is working with the mRNA of this mutated polypeptide. She labels the codon carrying the mutation as codon 0, so that the subsequent codons would be 1, 2, 3 and so on. She realizes that she can undo the mutation and produce the same wild type polypeptide if she...

1. deletes the mutated base,
2. pulls out the second base of the stop codon of the mRNA and inserts it right in between the first two bases of codon 1.

(a) Identify the amino acid 'codon 1' codes for. (you will need to use your answer to 1.5)

(2 points)

(b) The net effect of the two steps is deletion of a base, yet frame shift mutation is not observed in the mRNA. In **not more than 70 words**, explain why.

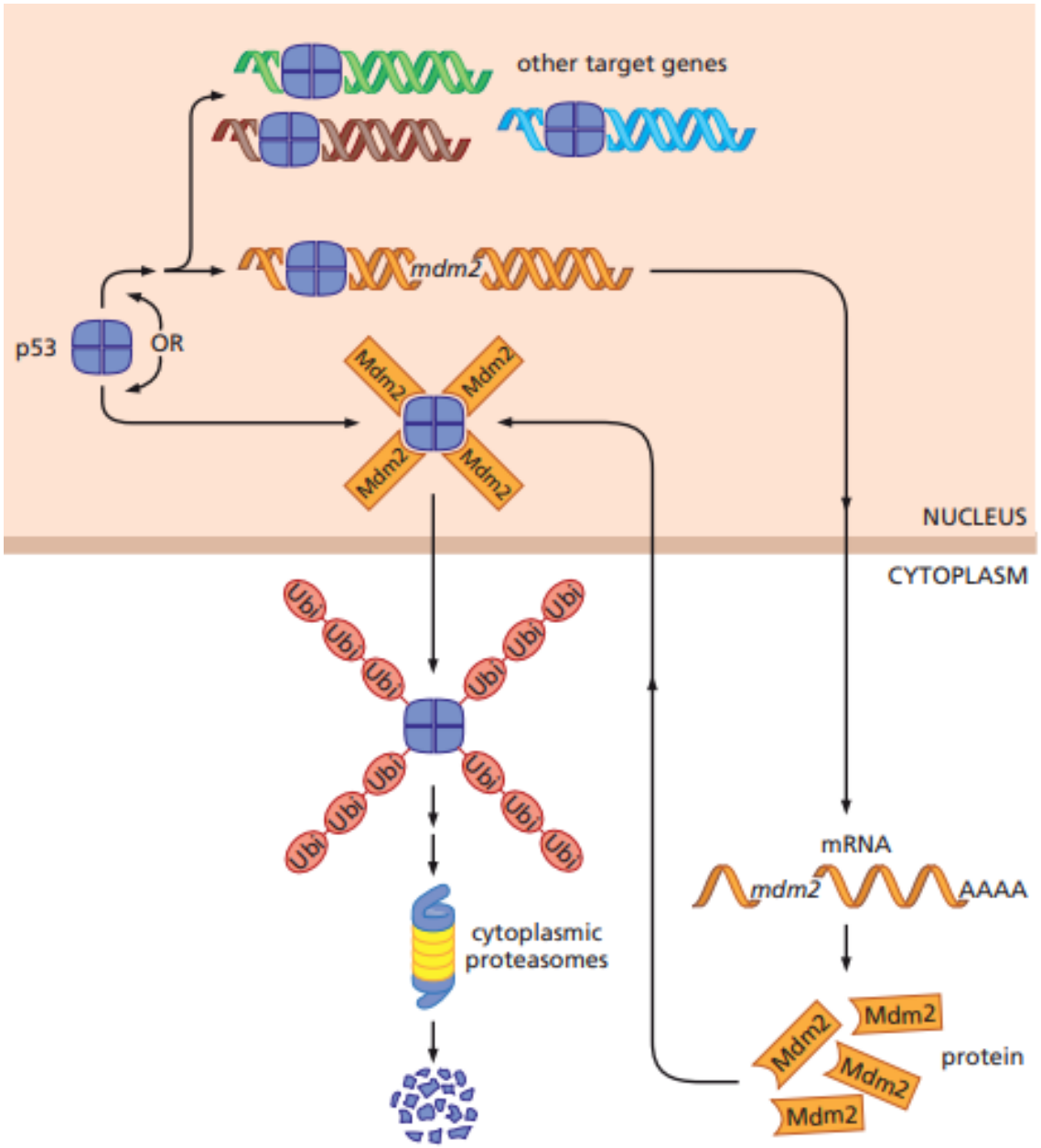
(3 points)

1.8

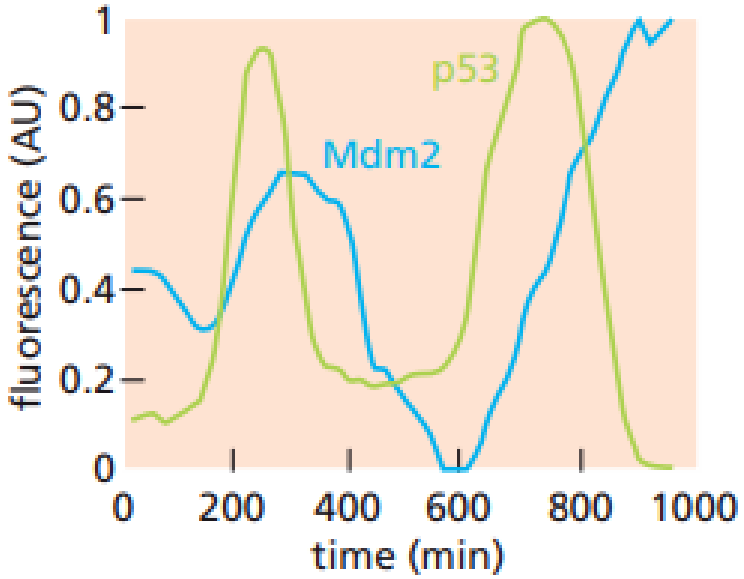
p53 is a critical protein in the cell cycle which gets activated during genotoxic assaults to the cell. p53 is inactivated in most cancer cells. Answer the following questions on p53:

(a) p53 is a tetrameric protein. All 4 subunits are crucial to its functioning.
I take a normal healthy growing cell line. In one subset of cells (labelled A), I introduce a point mutation in only one p53 allele. This mutation destroys functionality of the protein encoded by that gene. The other allele is normal. Hence, the genotype is $p53^{+/-}$.
In another subset of cells (labelled B), I knock out one of the p53 alleles. The genotype is $p53^{wt/null}$.
Which of the cell lines would score positive for tumorigenesis in soft agar colony formation assay?
Justify your answer schematically or in **not more than 2 sentences**.
(1 + 2 = 3 points)

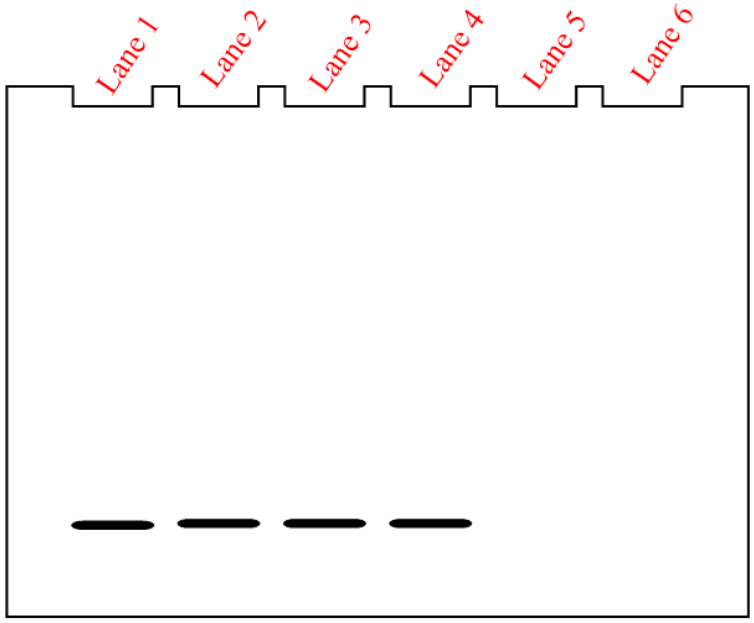
(b) Consider the following signalling pathway.



Also consider the following graph. The fluorescence axis refers to the fluorescence intensity reported from the fluorescent tags conjugated to p53 and Mdm2 protein.



Furthermore, a Northern Blot analysis was performed on the total RNA extracted from a mammalian cell. The fragments were hybridized with a probe complementary to the sequence for p53 RNA.



The lanes correspond to the following:
Lane 1: $p53^{+/+}$ cell line, RNA extracted just after starting culture
Lane 2: $p53^{+/+}$ cell line, RNA extracted 200 minutes after starting culture
Lane 3: $p53^{+/+}$ cell line, RNA extracted 400 minutes after starting culture
Lane 4: $p53^{+/+}$ cell line, RNA extracted 600 minutes after starting culture
Lane 5: $p53^{-/-}$ cell line, RNA extracted just after starting culture
Lane 6: $p53^{-/-}$ cell line, RNA extracted 400 minutes after starting culture

(I) State whether the following statements are true or false:

(i) Mdm2 and p53 are involved in a mutually inhibitory negative feedback circuit.

(ii) p53 has a short half-life in the cell.

(iii) The short half-life of p53 is not due to control at transcriptional level but post-translational regulation.

(iv) Mdm2 can act as oncoprotein.

(1 × 4 = 4 points)

(II) Many cancer cells which carry defective mutant p53 have been observed to produce very high concentrations of the protein. Given the tumour suppressive role of p53, explain this counter-intuitive and paradoxical observation using **only** the information given above.

(2 points)

(III) p53 phosphorylation can stabilize the protein from degradation. I have identified a kinase which functions to increase the p53 level in a cell. I incubate p53 protein isolate with the kinase, and then carry out a Western blot with an anti-phospho p53 specific antibody. No bands are detected in the lane in which kinase is added. Based on whatever is provided about p53 in this question, propose a mechanism as to how the kinase could then increase p53 levels in the cell.

(3 points)

1.9

A 30-year-old woman, at 28 weeks' gestation comes to the office for a prenatal visit. She has had one previous pregnancy resulting in a spontaneous abortion at 12 weeks' gestation. Today, her vital signs are within normal limits. Physical examination shows a uterus consistent in size with a 28-week gestation. Fetal ultrasonography shows a male fetus with no abnormalities. Her blood group is O, Rh-negative. The father's blood group is B, Rh-positive. The physician recommends administration of Rho(D) immune globulin to the patient.
This treatment is most likely to prevent which of the following in this mother?

(i) Development of natural killer cells.

(ii) Development of polycythemia.

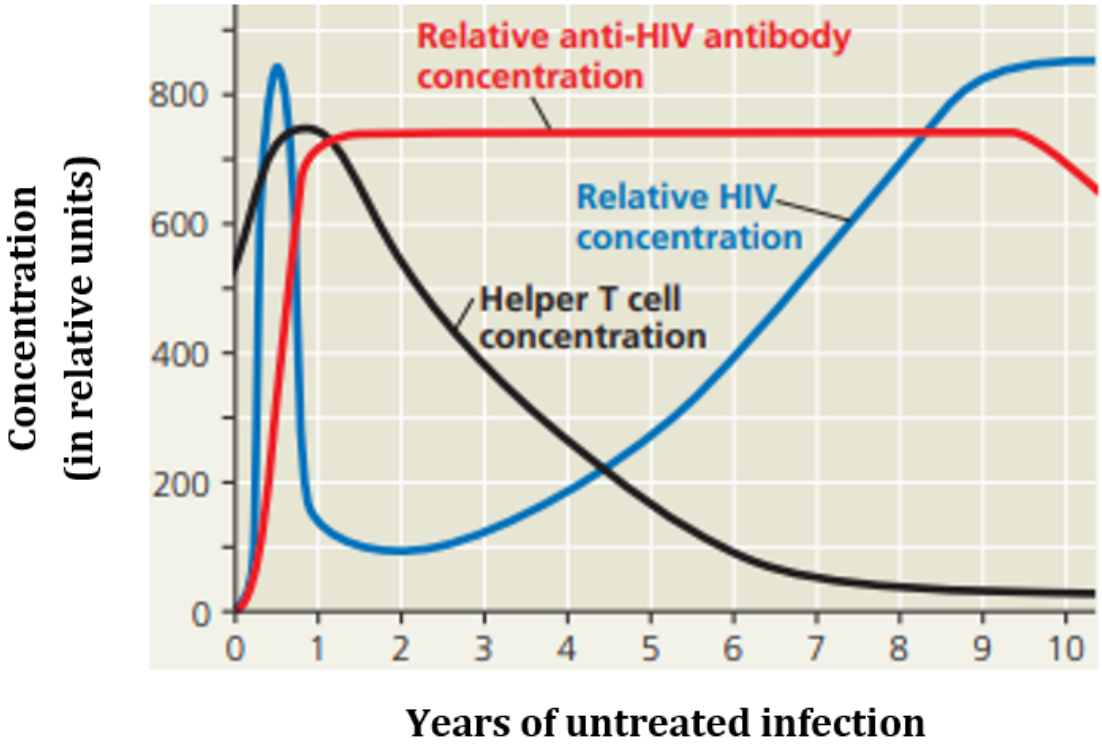
(iii) Formation of antibodies to RhD.

(iv) Generation of IgM antibodies from fixing complement in the fetus.

(v) Immunosuppression caused by RhD on erythrocytes from the fetus.

(*2 points*)

1.10



Consider the graph above. It represents the relationship among the serum concentrations of HIV virus particles, helper T cells, anti-HIV antibody in the serum of an individual upon infection by the virus.
Based on these graphs, answer the following questions:

(a) What is the primary reason for the drop in HIV virus particles in the serum towards 6-12 weeks post infection?

(*1 point*)

(b) What can be the best explanation for the rise in the number of HIV virus particles after 1 year of infection?

(*1 point*)

(c) If you are to draw the concentration of $CD8^+$ T cells on the same graph, what would it look like?

(*1 point*)

(d) What is the primary reason for the dip in the concentration of anti-HIV antibodies after 9 years?

(*1 point*)

(e) A person's blood serum is tested using ELISA. The report mentions that HIV has not been 'detected' in the person. The person produces this report in a blood donation camp and wishes to donate 2 units of blood. Should the medical personnel in the camp allow the person to donate blood?

(*2 points*)

1.11

Aditya has isolated two species of bacteria and brought them to me. I have labelled them as species A and species B. On doing a detailed sequence analysis and comparing with available genomic sequences, I conclude that these two species have been already reported in literature and are known to be mutualistic. I now want to culture each of the species individually and also perform a co-culture and plot the growth curves. Draw the expected growth curves when...

(a) A is grown individually.

(b) B is grown individually.

(c) They are grown in co-culture (draw growth curves of both the species on the same set of axes in this case).

When co-cultured in liquid medium and then plated, colony morphologies of A and B are very distinct. Hence, there is no problem in isolating each of the species from a co-culture and obtaining its individual growth curve when grown in the vicinity of the other, hence, question (c) above is not unfeasible to carry out experimentally. Remember to put some rough numbers on the axes so that we have a sense of the relative rates of growth of one species when grown in isolation and when grown in presence of the other.

(*1 + 1 + 1 = 3 points*)

1.12

Mark takes a genetic screening test and is told that he is affected by A. He promptly asks his wife to take a test to find the probability of their next child inheriting this trait. The lady, however, read a social media post that said genetic screening is dangerous to health and refuses to take it, come what may. As a last resort, Mark screens her parents' genotypes and discovers that her mother is a carrier while her father is unaffected. Given that Mark and his wife have 3 sons and 2 daughters, all

normal, what is the probability that their next child is an A-affected son?

(*4 points*)

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2 (*45 points*) Spongy Brain

2.1

Prion diseases are caused when a protein in the body called PrP^C is misfolded due to small changes in its structure to the abnormal variant PrP^{Sc}, which then goes on to infect other PrP^Cs. This generates long polymers of self-growing proteins called 'prions' which cause many degenerative diseases in both humans and animals. The conversion process from the normal protein to its abnormal is very complicated and involves a lot of unknown reactions.
PrP^C contains a region in its core with the amino acid sequence of the form ZXXXZ where Z is a common amino acid in the repeated sequence and X denotes various hydrophobic amino acids. This 'Z' amino acid rich region forms an α-helix and is embedded in the membrane (PrP^C is a transmembrane protein). One of the main factors which causes conversion of PrP^C to PrP^{Sc} is thought to be the transformation of shape of the 'Z'-rich region to a β strand.

(a) What could the amino acid Z be and why? Why does conversion to β strand help in making prions infectious?

(*2 + 2 = 4 points*)

(b) How would you check if it's this region that has changed its structure when it converts to PrP^{Sc}? What sort of experiment can you perform?

(*4 points*)

(c) Now you decide to test if it's actually the amino acid 'Z' which is the deciding factor for the change in the secondary structure. How would you conduct an experiment to do this?

(*4 points*)

2.2

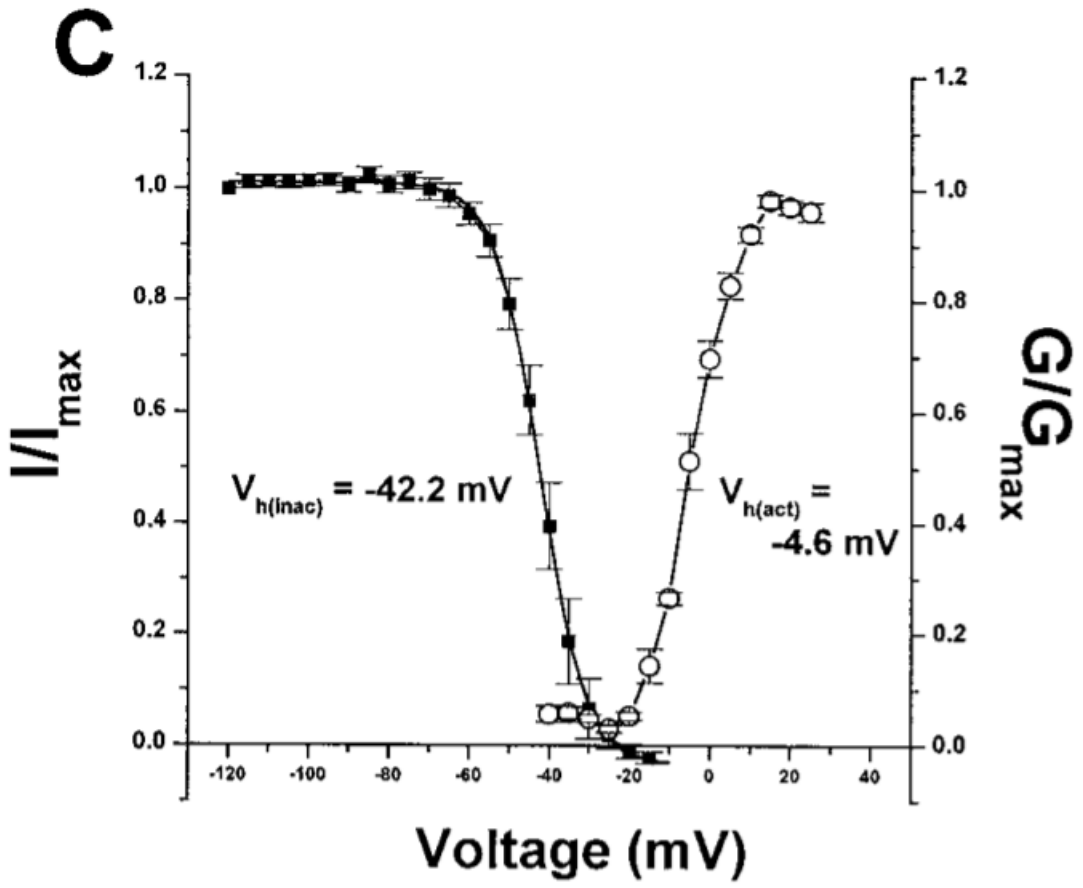
Prions are known for causing many neurological diseases. To know more about this, we decide to conduct an experiment in which we add the 'infectious' part of the prion (or a subpart of it) mentioned above into a neuronal extract. We now want to measure it's effect on the current through various ion channels.

(a) The two main contributors to the action potential are the sodium and potassium ion channels (actually a specific type of potassium ion channel) . Knowing the mechanism of action potentials, can you roughly plot the currents (just the shape of the graph, the actual values of current are not

required), say I_{Na} (current through the sodium channel) and I_K (current through the potassium channel) with respect to the potential V of the cell when the cell is excited. Indicate the resting potential V_r (-70 mV) and the threshold potential V_t (the upper range of the voltage in the graph should be of the order +30 mV). Give a very brief explanation as to why the graphs must be so.

(3 + 1 + 3 = 7 points)

(b) There are many types of potassium ion channels which can be present in a neuron. One of them has the current (say I_A) vs. voltage graph as follows:



I_{max} is the maximum current through the channel, G is the conductance of the membrane, with G_{max} being the maximum conductance. There are two different curves combined in the graph, one whose points are indicated by black squares and the other by hollow circles. The voltage was changed from -120 mV to -15 mV for the first one while it was increased from -60 mV to 20 mV for the second one. These represent the curves for inactivation and activation of the channel respectively. The voltages for half inactivation and half activation are represented on the graph

Based on this, could you speculate what the channel's function is?

(*2 points*)

(c) To study the effect of the prion protein segment on the activity the channels, we decide to measure the currents passing through these channels at various voltages. But as it turns out, we can only measure the total current passing through the entire membrane of the neuron. How can we modify the experiment to get a specific channel current of our choice (we assume that current in each channel for a particular voltage is independent of the others).

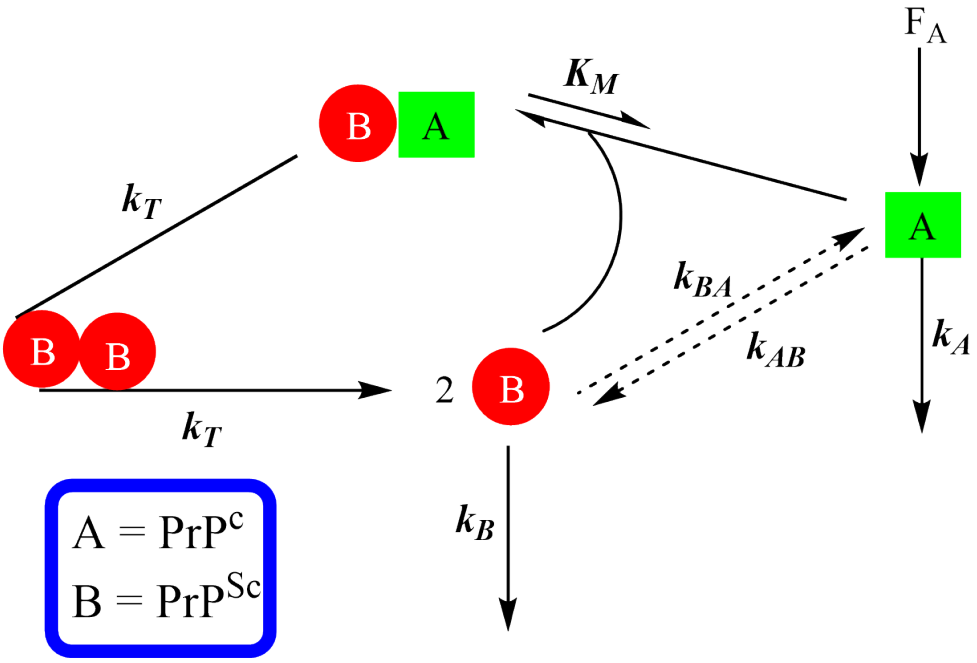
(*3 points*)

(d) The results then show that there is a downward shift in the I_K vs. voltage and I_A vs. voltage graphs whereas the graph of I_{Na} vs. voltage is largely unchanged. What effect does this have on the normal functioning of a neuron?

(*2 points*)

2.3

A simple model was proposed initially for the formation of PrP^{Sc}, call 'B' molecules from PrP^{C} molecules, call 'A'. A schematic for the model is depicted below:



The processes and the corresponding rates are ([A] denotes concentration of A):

Process	Rate
Non-catalysed conversion of A to B	$k_{AB}[A]$
Non-catalysed conversion of B to A	$k_{BA}[B]$
Decomposition of A	$k_A[A]$
Decomposition of B	$k_B[B]$
Rate of formation of A	S_A
Auto-catalysed formation of B	?

(a) If the Michaelis constant is K_M and the turnover rate (catalysis rate) is k_T, what is the rate of formation of B which is catalysed by itself? (Assume that this obeys Michaelis-Menten kinetics, similar to enzyme catalysis)

(4 points)

(b) Differential equations are equations which involve the derivatives of a variable (change in the value of a variable for very small changes in another variable). In biology we are mainly concerned with the rate, that is the change with respect to time. It's written as

$\frac{d[X]}{dt}$ = (Rate at which X is made) - (Rate at which X is used up/destroyed)

Using the table, write the expressions for $\frac{d[A]}{dt}$ and $\frac{d[B]}{dt}$

(2 + 2 = 4 points)

(c) Steady states are attained when the concentration of the species involved does not change with time? What are the equations for the steady states (you do not have to solve them)? Why will there be two steady state solutions?

(2 + 2 = 4 points)

(d) One steady state concentration is:

$$[A]_1 = \frac{S_A}{k_{AB} + k_A}$$

$$[B]_1 = \frac{[A]_1 k_{AB}}{k_{BA} + k_A}$$

The other steady state concentration is:

$$[A]_2 \approx \frac{k_B}{\frac{k_T}{K_M}}(1 - \frac{k_A k_B}{S_A \frac{k_T}{K_M}})$$

$$[B]_2 = \frac{S_A}{k_B}(1 - \frac{k_A k_B}{S_A \frac{k_T}{K_M}})$$

Which one of these steady states (1 or 2) corresponds to the state when this mechanism of infection is effective? Give (mostly qualitative) arguments by making reasonable assumptions about the value of the constants.

(2 + 5 = 7 points)

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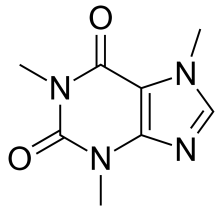
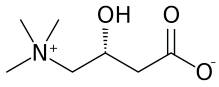
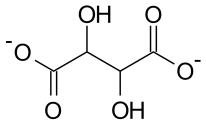
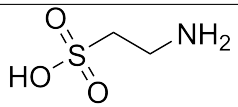
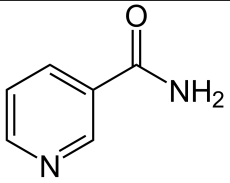
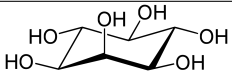
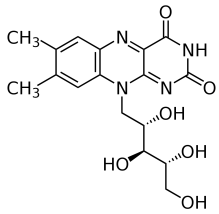
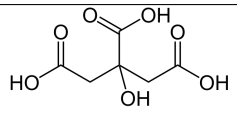
3 *(23 points)* A "Monster"ous Antibiotic

You have an exam tomorrow morning and you have no idea what was taught all semester. Naturally you are panicked and plan to pull an all-nighter and finish the syllabus (at least partially) so that you don't miserably fail. Your roommate takes out yet another can of an energy drink hoping it will get the two of you through the night and then perhaps another for staying up during the exam. Seeing the excess consumption of energy drinks, you are worried about your roommate. You ask them to limit their excess consumption of energy drinks as anything in excess can be harmful! Your roommate agrees, however, having worked in a laboratory which is exploring alternatives to antibiotics, something more natural, to counter the rising threat of antimicrobial resistance, your roommate tells you that energy drinks may actually be able to prevent bacterial gut infections! You are bewildered, as of course anyone would be! Energy drinks for anti-bacterial use? Your roommate starts explaining it to you, however, you are not able to catch all the technical details. Suddenly you remember about the exam and start studying again.

Next day after the exam you try to recollect what your roommate told you. You remember the following about the research that was done:

> "Aerobic pathogenic bacteria in the gut ..... When gut microbiota is present, it confers additional resistance to the bacteria upon secondary infection ..... The mechanism is due to a component in bile juice (A) ..... The concentration of this component was high after infection ..... in mice with gut microbiota, the gut bacteria were able to metabolise this compound and clustered around it ..... One of the metabolites produced (B) was able to block an enzyme (C) in the pathogenic aerobic bacteria which is vital to its functioning."

You pull out your last can of Monster® and check the ingredients. You see the following:

Ingredient	Structure (known to you)
Caffeine	
L-Carnitine	
L-Tartrate	
Taurine	
Niacinamide	
Inositol	
Riboflavin	
Citric Acid	

In addition, you have the following information:

(i) A is considered to have a role as a neurotransmitter

(ii) B has a role in reducing blood pressure

(iii) C contains both copper (Cu) and iron (Fe)

(iv) In addition, you know the following about the metabolic pathway of A:

- In different bacteria, the metabolism proceeds differently

- In one type of metabolic pathway, the following products are obtained:
  - ☐ In Bacterium P, the products are: Acetate + NH_3 +B
  - ☐ In Bacterium Q, the products are: Acetate + NH_3 + ${S_2O_3}^{2-}$
- In another metabolic pathway, Three enzymes, X Y and Z are involved. These convert A to sulfoactaldehyde. Among the three, X and Z are commonly found in respiration and have paired functions. Y is named as *A_____ase*, where A stands for the name of the compound A and the blank followed by '*ase*' is representative of the function of the enzyme. The sulfoacetaldehyde is broken down further, of which one of the products is B.

(v) Also, you have the following information about the relevant component of the energy drink:

- It is an organic acid
- It is considered an amino acid with anti-oxidant properties

Your job is now to decipher the mechanism of the defense against the pathogenic aerobic bacteria. You need to answer the following:

3.1

What is the component A and its metabolite B?

(2.5 + 2.5 = 5 points)

3.2

What are the enzymes X, Y and Z? To answer for Y, you need to answer it as *A____ase*, by substituting for the name of A and the function of the enzyme.

(1.5 + 1.5 + 1.5 = 4.5 points)

3.3

What is the name and function of the enzyme C? How does B block the enzyme?

(3 + 1.5 = 5.5 points)

3.4

Where does the energy drink come in this entire narrative? How might an Energy drink help in the defense against pathogenic bacteria? (Note: the use of Energy drink is only a claim here and its consumption as an alternative antibiotic is not recommended. This is a normal fun-stretching-the-possibilities-of-scientific-studies conversation between you and your roommate)

(5 points)

3.5

What result would be expected in this pathway if the test subject would have had an antibiotic treatment prior to the experiment?

(*4 points*)

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4 (*30 points*) Might-o-chondria

4.1

Consider the hydrolysis of ATP to produce ADP and inorganic phosphate. This involves breakage of a bond. I am way too curious about ATP. I have synthesised ATP in the laboratory and want to carry out experiments on ATP in the gas phase. Assume that there exists a chemical reagent X (might even be an enzyme) which can convert ATP into ADP and Pi. Furthermore, assume that I am carrying out single molecule experiments in which I have irreversibly attached X to a glass slide. I am now incubating it with ATP molecules in the gas phase. There are no traces of moisture in the set up. X will cleave ATP into ADP and Pi. The nucleophilic attacking moiety is contained in X itself, and one molecule of X can carry out only one round of this cleavage of ATP.
Will the hydrolysis of ATP lead to a net gain in energy in this experiment? Justify your answer.

(*1 + 2 = 3 points*)

4.2

I plan on carrying out an interesting experiment as follows:

(i) Isolate mitochondria

(ii) Get mitochondrial membrane fragments along with the transmembrane proteins

(iii) Incubate with a mixture of enzymes

(iv) Add specific substrate

(v) Assay for the function of particular components in the Electron Transport Chain

Answer the following questions related to this experiment:

(a) How can step (i) be carried out? Answer in **not** more than **one sentence**.

(*1 point*)

(b) Can you think of a method to carry out the second step in the sequence above?
(*2 points*)

(c) Suppose I incubate the mitochondrial membrane isolate obtained in step (ii) of the experiment with α-ketoglutarate and succinyl CoA synthetase. What are the expected observations if I assay for the amount of O_2 utilized?
(*2 points*)

(d) I repeat the same experiment as before. However, I now carry out the experiment not with mitochondrial membrane isolates but with whole mitochondria. This time I add α-ketoglutarate only (no extra enzyme added). You're now performing a spectrophotometric assay very rapidly for the amount of reduced cytochromes in aliquots of the mixture at regular time intervals. How would the graphs for the amount of reduced cytochromes vs. time look like? Remember to LABEL your axes **properly**. Draw separate graphs for each of the cytochromes using the **same set of axes**. Explain why you feel that the plots would look like the way you have drawn.
(*6 points*)

(e) Say now I reconstitute a vesicle with a mitochondrial membrane (along with all the embedded proteins). I add α-ketoglutarate then. I am performing the same rapid spectrophotometric analysis for the amounts of reduced cytochromes and plotting their changes with time. Since this is a reconstituted vesicle, the interior of it just contains buffer. What would the graphs look like now? Would it be any different if succinate was used instead of α-ketoglutarate? Justify your answer **briefly**.
(*3 + 2 = 5 points*)

4.3

Inhibitors have provided extremely useful tools for analyzing mitochondrial function. Mitochondria were added to a phosphate-buffered solution containing succinate as the sole source of electrons for the respiratory chain. After a short interval, ADP was added followed by an inhibitor. Draw the graph for the amount of oxygen consumed vs time if the inhibitor added is FCCP. What would the graph look like if the two inhibitors are added to the same reaction mixture – oligomycin followed by FCCP and in another case malonate followed by cyanide? Justify the plots and rationalize the differences or similarities between the three plots.

Inhibitor	Function
1. FCCP	Makes membranes permeable to protons
2. Malonate	Prevents oxidation of succinate
3. Cyanide	Inhibits the cytochrome *c* oxidase complex
4. Atractyloside	Inhibits the ADP/ATP transporter
5. Oligomycin	Inhibits ATP synthase

(1 + 2 + 2 + 6 = 11 points)

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5 *(23 points)* "Unity is Strength" - but why?

5.1

A new species of bacteria was recently discovered by scientists. The bacteria are colonial and the colonies observed are also not very large. The bacterial colony is covered by a sheath. When some individuals in the colony sense favourable conditions for growth such as availability of nutrients, etc , they secrete a communication molecule into the space between the cells and the sheath. The rate of respiration in the individuals that secrete this molecule increases. The sheath covering the colony is impermeable to the signalling molecule. When other bacteria in the colony receive this molecule, they take in potassium ions using a potassium-proton exchange protein. The communication molecule has a molecular weight of around 450 Da.

(a) Is the increased rate of respiration of any significance for the intercellular communication using the signalling molecule? Why/Why not? Why does potassium influx occur in the bacteria which receive the molecule?

(1 + 2 + 2 = 5 points)

(b) Which of the following best describes the signalling molecule? Justify with respect to the significance of the structure in the signalling mechanism. Any option followed by a coherent logical justification will fetch you full credit.

(i) Contains one secondary or tertiary amino and one acid group.

(ii) Contains two amino (secondary/tertiary) groups.

(iii) Contains a carboxylic acid and a conjugated amino (secondary/tertiary) groups.

(iv) Contains two acid groups.

(1 + 3 = 4 points)

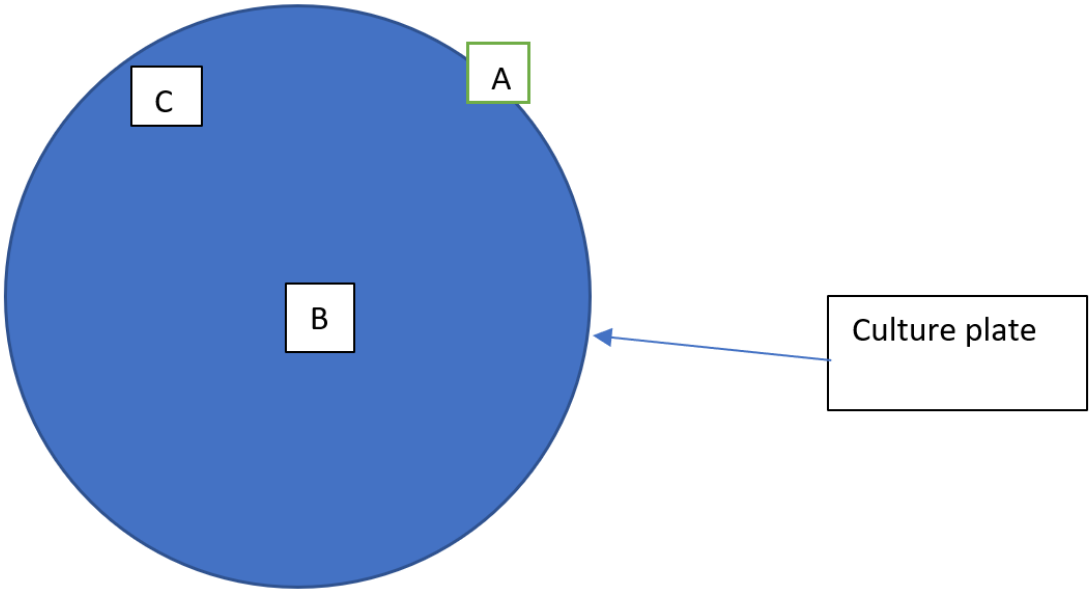
(c) Is a mechanism to prevent the re-entry of the signalling molecule into the bacteria that secrete these molecules necessary? If yes, explain one such possible mechanism in one or two sentences. If no, give a brief explanation. *(1 + 3 = 4 points)*

5.2

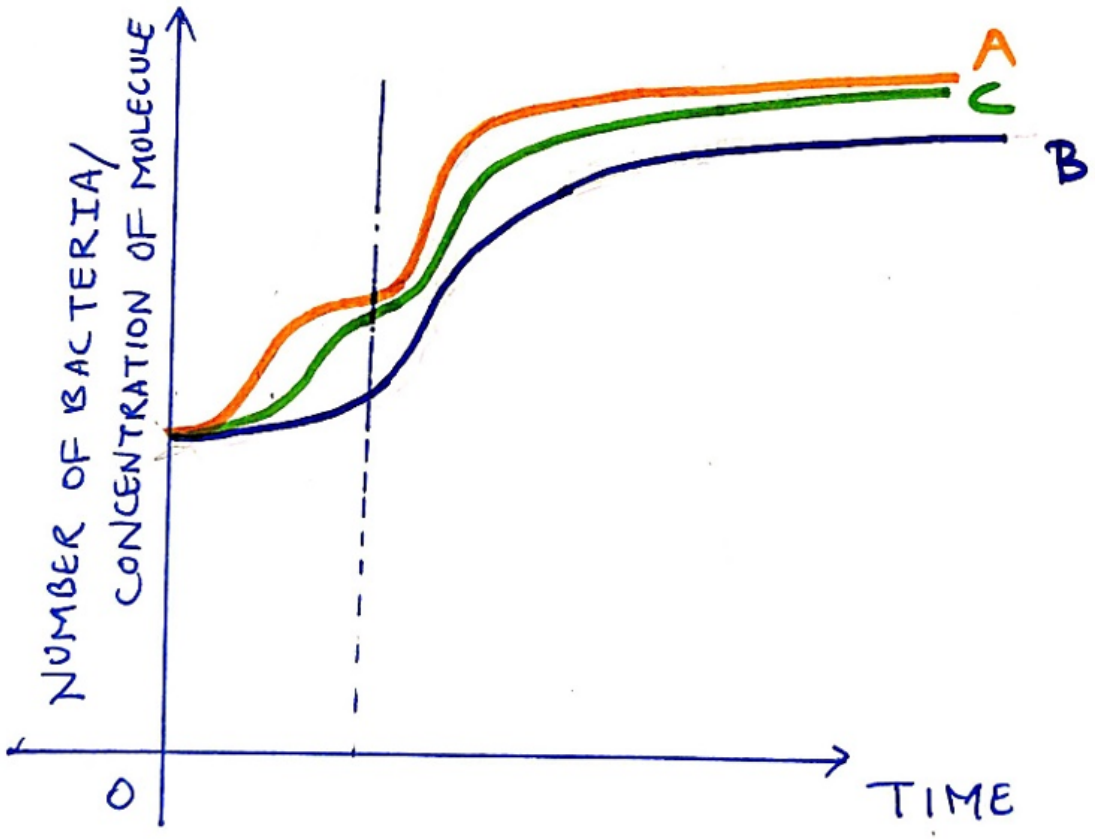
A bacteriologist, Mr. Xu, collects a sample of bacteria for analysis in his lab. Metagenomics indicates that the sample contains only one species of bacteria. Assume that the sample contains only one species of bacteria.

(I) The sample is cultured, and an aliquot of this culture is plated on a culture plate. A bacterial lawn is observed. So, the scientist repeats the plating, but finds that all plates have a lawn. Xu tries to serially dilute the sample and plate it, but even this fails to work.

(II) A portion of the sample is cultured in soft agar medium put in a petri dish. The sole carbon source in the medium within the dish is glucose. Xu also wishes to assay for glucose in the culture medium. While preparing the soft agar, he has mixed traces of a dye which given blue colour when glucose in present in the medium and becomes colourless when glucose has been totally exhausted. The dye is non-toxic to all the bacterial cells.
To simulate a realistic situation, after the blue colour has been completely lost, the petri dish is kept in a bath containing a liquid medium containing lactose. The lateral walls of the plate are porous! However, molecules can diffuse through the walls but not bacteria. The lateral walls are the only walls of the plate through which diffusion between the media inside and outside the plate can occur. Assume that the diffusion of lactose through soft agar is slow enough for effects of changes in its concentration to be detectable in real time. The concentration of all substances is the same in the medium within the petri dish and the medium outside the petri dish, except for the presence of glucose in the former and lactose in the latter. Assume that oxygenation is uniform throughout the plate and the species is aerobic. Samples are regularly collected from site A, B and C after keeping the plate in the "bath" and the growth curves are plotted on the same graph.

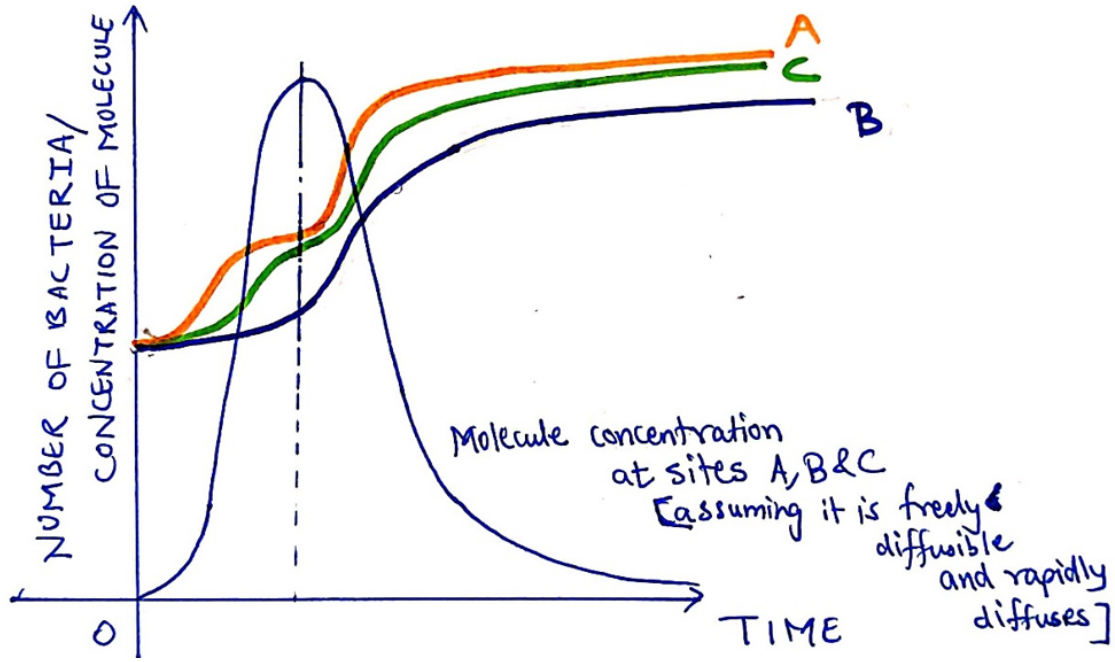


Please note that **all distances are to scale** in the figure above.
Site A is at the periphery of the plate, B is almost at the center and C is slightly away from the periphery but far away from the centre too.



(III) The species of bacterium is identified. Strangely, during the initial part of the second growth phase of the curves obtained in (II), a molecule that the species produces on exposure to stress is found in the three samples collected

from sites A,B and C. However, it is found that the concentration of the compound is high during the initial part of the second growth curve but reduces as growth proceeds.



(IV) When the sample was grown in a continuous culture system, on providing lactose, the concentration of the molecule increased and remained high and the growth of bacteria was uniform at all the sites A,B and C.

Think about all the four observations. What would you infer from each of these four observations? Explain what might be going on with the help of the inferences that you have drawn. Any logically correct explanation will be awarded full credit. It might be easier to answer this question if you brood over concepts of microbial ecology, like social cheating, quorum sensing, etc.

(*10 points*)

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6 (*30 points*) Opera-On!

6.1

What's magical about neural systems is that they're one of the best engineered systems in nature; its complexity is something that we can only aspire to reach – oh wait, we're already working towards it! We've discovered biological equivalents to electronic devices even in bacterial systems: after all, engineering ideas are

inspired by nature.
On a cellular operon level, I find beautiful analogues to electronic circuits; it's unbelievable, I might have stumbled on something groundbreaking here. I realise I've been rambling without introducing myself; I'm Shanaya, an aspiring synthetic biologist. I work with the [hypothetical :)] bacterium species *Vibrio blitzius*, an unconventional choice for a biologist, sure, but an easily exploitable microbe once we work its operons out. I've unraveled a basic idea of the operons of this bacterium:
It seems to have a novel feature that its cousins lack: a special group of operons that are preferentially activated in extreme environmental conditions, producing a seemingly uncanny protein that activates the operon which in turn activates endospore-formation. The protein is a story for another time.
The bacterium that I work with is absurdly altruistic; above its quorum, it has a programmed cell death feature coupled with how extreme the conditions are to it. For example, humidity loss might not be a big factor leading to programmed death or endospore formation, but coupled with high temperature, its effect is much more pronounced and unavoidable in our analyses.
The operons detect extreme conditions through a cascade of speedy biomolecular reactions involving a ton of enzymes known to every biologist. Now, I have a task in front of me: I have limited lab exposure due to the ongoing pandemic, so I'm left to theorizing everything about this whole mechanism of bacterial endospory (let's call this one Operon E) and cell death (let's call this one Operon CD) before I have my final opportunity to test my hypotheses. As far as I'm aware, these operons must work together to generate an output that activates another operon (let's call this one Operon P) controlling proteins needed to form an endospore. I need to figure out how the operons work together by providing an analogous digital logic circuit. Could you help me out in sketching a highly simplified model?
Remember that the apple doesn't fall far from the tree. There's beauty in elegance, truth in simplicity.

(*9 points*)

6.2

All's well and good now, and by the proteins extracted from the bacterium, I've hypothesized the DNA sequence of Operon P. However, when my colleague performed gene sequencing for this bacterium (the operon is a small one, remember), some of the codons didn't match my model. I'm baffled, as the other *Vibrio* sp. obey the pattern of codons pretty well. Why did I observe a difference? Could this disrupt the taxonomic order of the bacterium? If you think about it, there could be an evolutionary implication too...

(*2 + 2 + 2 = 6 points*)

6.3

The ability to incorporate chemically diverse nonstandard (i.e. unnatural) amino acids (nsAAs) in polypeptides synthesized by a bacteria broadens the structural and functional diversity of its proteins. nsAAs with varied sidechains can serve a plethora of functions of biological interest. Using SynBio techniques, I've genetically modified one of the plasmids (let's call it pGOOD) that *V. blitzius* possesses, intensifying the need for its biocontainment. I plan to utilize an approach called **"synthetic auxotrophy"**, where I introduce nsAA dependency in an essential protein or its subunit. I engineer the incorporation of nsAA biphenylalanine (BipA) in the context of stop codon UAG. Note that UAG is not the naturally found stop codon in the genes encoding DNA polymerase III subunits, and the subunits are essential for the proper functioning of the protein. ALso, DNA pol III is crucial for the survival of *V. blitzius* host.
To achieve my goal (i.e. synthetic auxotrophy), I follow the following steps sequentially:

(i) I make the host's entire genome devoid of any UAG codon (of course in the relevant reading frame).

(ii) I transform the host with a plasmid which has the gene called $holB^r$) cloned in it. The gene $holB^r$ is a slightly modified version of *holB* gene, and the *'r'* in superscript denotes codon **r**epurposing. The gene *holB* in our native host encodes for DNA pol III subunit δ. The altered version contains an extra codon compared to the wild type subunit: just an added UAG upstream of the functional domain of this subunit and just after the start codon.

(iii) Site-specific incorporation of BipA requires a dedicated aminoacyl-tRNA synthetase (aaRS)-tRNA pair, also known as an **orthogonal translation system (OTS)**, which must not cross-react with the host's native tRNA's and aaRS's. I take a native aaRS specific for phenylalanine (i.e. a standard AA) and then engineer its substrate specificity (i.e. recognizing BipA while discriminating any other AA in the cell) through rounds of computer-aided enzyme redesigning.

However, at the end of the above scheme I notice that the resulting BipA-associated aaRS (henceforth abbreviated as BipA-RS) is quite promiscuous. In other words, BipA-RS fails to effectively discern between BipA and the standard AA's :(.

(a) One of your colleagues suggests to you that the N-end rule pathway, which is known to be present in *V. blitzius*, can be utilized to design an *in vivo* quality control system based on the type of UAG incorporation I had adopted. Is it a logical suggestion? If yes, describe how it is supposed to work and provide the experimental workflow required to make this proofreading system more robust. Otherwise, devise a new quality control system and briefly mention

the principle behind it.

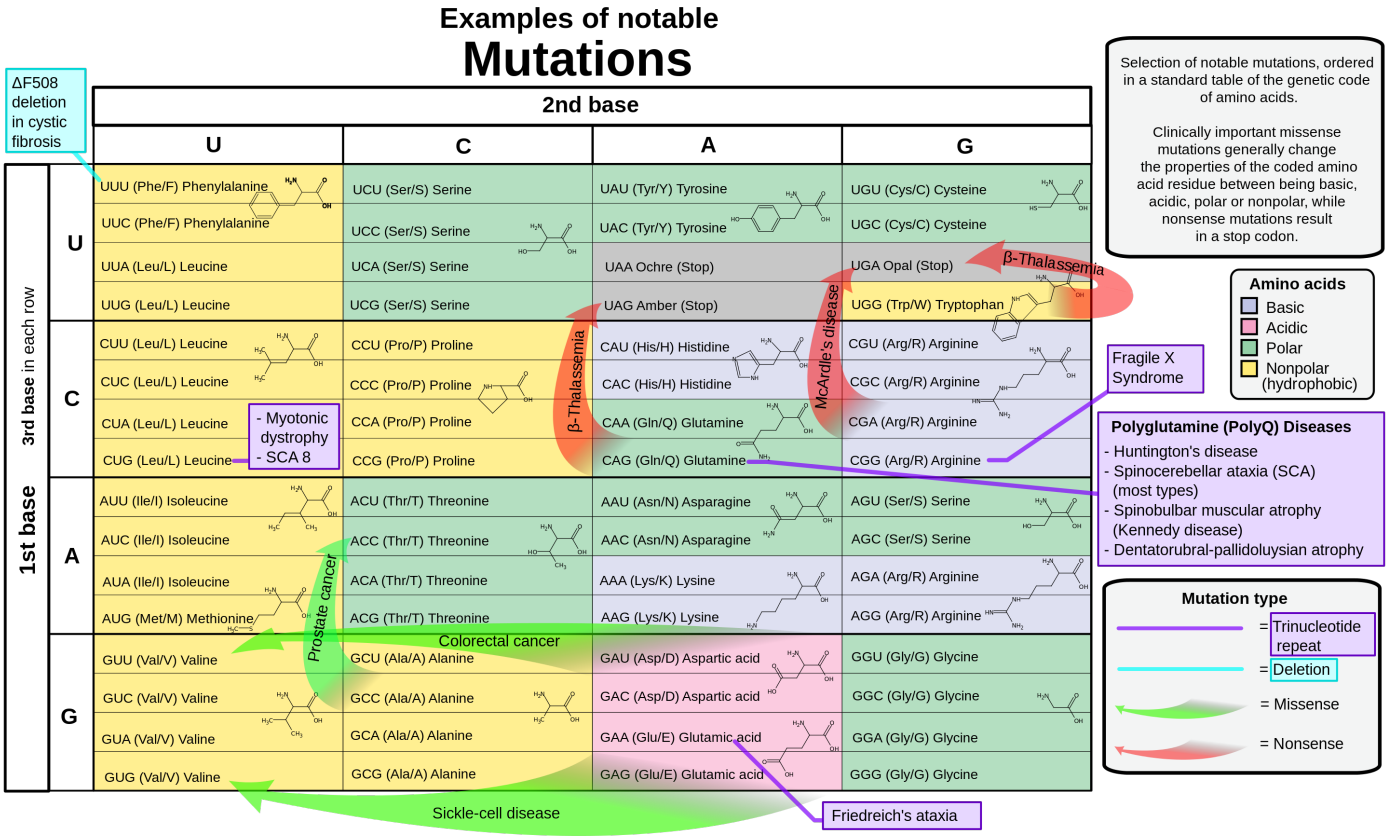
(1 + 8 = 9 points)

(b) While designing the sequence of $holB^r$, one can incorporate the repurposed UAG codon in-frame in many ways. Tell whether the incorporation strategy is valid or invalid in each of the following cases, from the viewpoint of biocontainment. Explain in brief in each case.

(3 × (0.5 + 1.5) = 6 points)

Where to incorporate the repurposed codon?		
Manner in which the repurposed UAG is being incorporated	**Validity of the strategy**	**Explanation**
One added UAG just after the start codon, and one added UAG in the tightly conserved functional domain of δ subunit	?	?
One added UAG at the third position starting from the start codon, and one added UAG in the region near C-terminal following the functional domain	?	?
One added UAG just after the start codon, and one UAG in place of a conserved alanine residue present in the functional domain of δ subunit	?	?

Standard genetic code



The End!