UNIVERSITY OF CAMBRIDGE

NST/BOC/WP
NATURAL SCIENCES TRIPOS Part 1A

Saturday 8 June 2019 9 to 12

BIOLOGY OF CELLS – PRACTICAL

*Answer **all questions.***

All questions carry equal marks.

Put each answer, including graph paper, into one or more answer booklets** and tie these up in a **single bundle.

***Yellow coversheet:** enter the numbers of each question you have answered and leave this loose on top of your pile of answer booklets. Please note that you should have answered **all** questions and this coversheet is only for confirmation.*

Write your examination number on each answer booklet cover.

STATIONERY REQUIREMENTS
Answer booklets (8 X 8 page)
Linear graph paper
Rough work pad
Tags
Yellow Coversheet

SPECIAL REQUIREMENTS
Approved calculators allowed

You may not start to read the questions printed on the subsequent pages of this question paper until instructed that you may do so by the invigilator.

For reference, a list of the questions is given below.
The average suggested time for each question is 15 to 20 minutes.

1. Microscopy
2. Protein structure
3. Enzyme activity
4. Photosynthesis and the oxygen electrode
5. Fungal genetics
6. Bacterial plasmids
7. PCR
8. Imaging cell division
9. The embryological zoo

1. Microscopy

You are given the specimens listed below (a-h) and asked to obtain images that will provide answers to the experimental questions that follow. For each specimen explain which microscopical technique you could use, and why. Include in your answer a brief description of how each sample would be prepared.

a) Amoeba, to understand amoeboid movement.

b) Pancreatic cells, to assess the distribution of nucleic acids.

c) New species of tardigrade (water bear), to reveal the animal's morphology.

d) "Cheek epithelial" cells, to study the distribution of ER using a specific fluorescent dye.

e) Pancreatic acinar cells, to observe the two nuclear membranes.

f) Cells of the giant algae, *Chara*, to study cytoplasmic streaming.

g) Pancreatic acinar cells, to observe the distribution of mitochondria.

h) Mouse epidermal cells from a strain expressing the protein tubulin fused to Green Fluorescent Protein (GFP), to understand cell division.

2. Protein structure

The structure of myoglobin, a 154 amino acid soluble cytoplasmic protein, is shown in Figure 1A. The prosthetic group of myoglobin is shown at higher resolution in Figure 1B.

A B

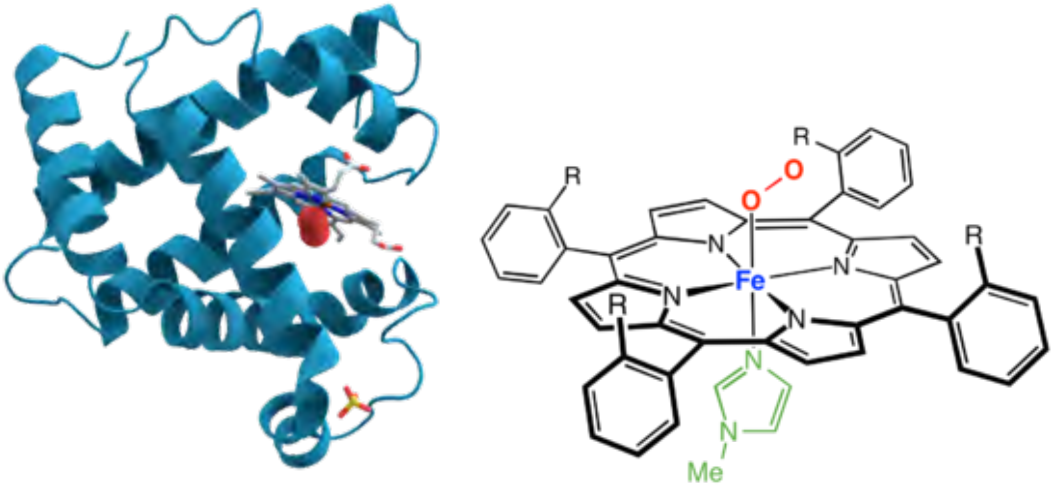

Figure 1. Structure of myoglobin (A) and its prosthetic group (B).

a) Name the secondary structures evident in myoglobin.

b) Name the prosthetic group found in myoglobin and its molecular function.

c) Name a second type of prosthetic group and an example of its molecular function.

d) Draw three amino acid side chains that are more likely to be found on the interior of myoglobin.

e) Draw three amino acid side chains that are more likely to be found on the exterior of myoglobin.

3. Enzyme activity

Figure 2 shows the general mechanism in enzyme kinetics involving a single substrate that leads to the Michaelis-Menten equation.

Figure 2
E = enzyme, S = substrate, ES = Enzyme-substrate complex, P = product.

a) Write the formula for the Michaelis-Menten equation.

b) What is V_{max}?

c) What is K_M in relation to V_{max}?

A student performs two reactions to study enzyme kinetics. In reaction 1, substrate (S) is in 10-fold excess over enzyme (E) at the start of the reaction, while in reaction 2 a much greater amount of enzyme was used.

d) Which of these two reactions are suitable for measuring the reaction rate for the Michaelis-Menten equation and why?

The Michaelis-Menten equation makes several assumptions about the reaction in Figure 2. For each of the following assumptions, explain why they are valid:

e) The reverse reaction E + P → ES can be ignored.

f) The product (P) can bind to the active site of the enzyme, but the formation of EP as a competitive inhibitor of ES does not affect the Michaelis-Menten measurement.

g) If a reaction, such as hydrolysis, involves water in addition to the substrate (S), then the reaction still behaves as a first order reaction.

A Lineweaver-Burk plot (also known as a double reciprocal plot) is often used as a linear transformation of the Michaelis-Menten equation.

h) Using the graph paper provided, plot the data in table 1 as a Lineweaver-Burk plot.

[S] (μ M)	v_o (μ M/sec)
0.2	0.17
0.4	0.29
0.6	0.38
0.8	0.44
1	0.5
2	0.67
3	0.75
4	0.8
5	0.83

Table 1

In addition to the reaction described above (data shown in table 1), you perform the same reaction, but with double the amount of enzyme. Draw a dashed line on your plot representing what you would expect this reaction to look like on a double reciprocal plot. Label this line X.

k) What should happen to V_{max} and K_M in this situation?

You also perform the reaction described above (data shown in table 1) with a competitive inhibitor. Draw a dashed line on your plot representing what this reaction should look like on a double reciprocal plot. Label this line Z.

l) What should happen to V_{max} and K_M in this situation?

i) From your plot, what are the V_{max} and K_M for this reaction? Include the units.

j) A linear graph makes it easier to identify the V_{max} and K_M, but from looking at the distribution of the data points on the graph you have produced, what might be a limitation of the Lineweaver-Burk method?

4. Photosynthesis and the oxygen electrode

You performed a high-throughput CRISPR-Cas9 screen to knock out genes in tobacco seedlings. Through this approach you identify a gene, *PSN1*, that when disrupted causes pale green seedlings that grow slowly.
To investigate the role of *PSN1* you decide to measure photosystem activity using thylakoid membranes purified from wild type and *psn1* mutant seedlings, using an oxygen electrode.
You use 4 grams of seedlings for each genotype and homogenize them in 10 ml of 0.4 M sucrose buffer. The homogenate is centrifuged at 3000 g and the chloroplast pellet is resuspended in 2.5 ml of sucrose buffer.

200 µl of the chloroplast suspensions were removed and added to 800 µl of acetone. 1000 µl of this was used to measure absorbance at 652 nm (A_{652}) using a spectrophotometer. Multiplying the A_{652} value by 2.9 can be used to calculate the concentration of chlorophyll in mg^{chl} ml^{-1}. The A_{652} measurement from wild type was 0.302 and *psn1* was 0.220.

a) Calculate chlorophyll amounts (mg^{chl}) in the acetone extractions from wild type and *psn1* seedling extracts.

b) Why is it important to measure chlorophyll amounts in the extracts?

100 µl of the resuspended chloroplasts were placed in an oxygen electrode containing KCN and the electron acceptor phenyl quinone. The final volume in the electrode chamber was 1 ml. The change in oxygen tension in the electrode over a time-course was measured.

These data are shown in Table 2.

Light/Dark	Time	Wild type (mV)	*psn1* (mV)
Dark	0	400	398
Dark	30	401	405
Dark	60	415	406
Dark	90	422	418
Dark	120	428	420
Light	150	552	440
Light	180	667	501
Light	210	772	543
Light	240	843	592

Table 2. Oxygen electrode measurements from wild type and *psn1* chloroplast extractions. Lights were turned on immediately after the dark measurement at 120 seconds. An electrode reading of 1 mV is equivalent to 2.55×10^{-4} O_2 µmoles ml^{-1}.

(TURN OVER for continuation of Question 4)

c) Calculate photosynthetic rate in wild type in µmole O_2 min^{-1} mg^{chl-1}.

d) Calculate photosynthetic rate in *psn1* in µmole O_2 min^{-1} mg^{chl-1}.

e) What can you conclude from these data about photosynthesis in *psn1* as compared to wild type with respect to photosystem I and/or II?

f) How would you prove that the *psn1* phenotype was caused by the disruption of the candidate gene and not due to a mutation in another gene?

5. Fungal genetics

After random mutagenesis of a Wild Type (WT) *S. cerevisiae* strain you select an interesting clone that cannot grow on medium containing glycerol as sole carbon source and is also unable to grow at 37 °C.

a) How would you investigate whether the two phenotypes are due to mutations in the same gene?

Your investigation reveals that the two phenotypes are due to mutations in different genes.

b) How would you investigate whether *each individual phenotype* is due to a mutation in one or multiple genes?

c) If the mutation that caused the inability to grow on glycerol medium was due to a mutation in the mitochondrial DNA, how many colonies unable to grow on glycerol medium would you expect to find after backcrossing the mutant strain with a WT and analysing the spores? Give a molecular explanation for your answer.

Once you have established that both phenotypes are caused by mutations in single genes (you call them *gly1* and *ts1*), you decide to map them. For this purpose, you set up a cross between the two strains listed in table 3:

Mat a, *ura3-53*, *gly1* and *ts1*	Your mutant strain: unable to grow on medium containing glycerol and dead at 37 °C; also unable to grow on medium lacking uracil
Mat α, *leu2-1*	"WT" strain unable to grow on medium lacking leucine

Table 3.

(TURN OVER for continuation of Question 5)

d) In order to obtain spores from this cross you need first to generate a diploid strain. How would you do this?

The diploid strain is cultivated and sporulation is induced. Random spore analysis gives the result summarised in table 4:

Ability to grow w/o uracil	Ability to grow w/o leucine	Ability to grow on glycerol	Ability to grow at 37 °C	Number of spores
yes	yes	yes	yes	7
yes	no	yes	yes	140
yes	yes	no	yes	23
yes	yes	yes	no	90
no	yes	yes	yes	5
no	no	yes	yes	30
no	yes	no	yes	22
no	yes	yes	no	90
no	no	no	yes	184
no	yes	no	no	130
no	no	yes	no	5
no	no	no	no	5
yes	yes	no	no	30
yes	no	yes	no	50
yes	no	no	yes	11
yes	no	no	no	43

Table 4

e) Do these data confirm that the mutations which confer an inability to grow on glycerol and thermo-sensitivity are on single genes? Justify your answer.

f) From these data, can you say which genes are linked?

g) Calculate the genetic distance between the linked genes (expressed in cM).

(TURN OVER)

6. Bacterial plasmids

Samples from the inspection of an Oxford restaurant were streaked onto L-agar plates. Colonies that grew on the plates were identified as *Escherichia coli*. To ensure a fair and independent investigation of this scandal, the samples were sent to Cambridge and given to Biology of Cells students for further analysis.

a) The samples were found to be resistant to both ampicillin and chloramphenicol. Describe what experiments you would carry out to determine whether the resistance genes are associated with the *E. coli* chromosome or whether they are plasmid-encoded.

b) Assuming that the resistance genes are plasmid-encoded, how would you distinguish between one doubly-resistant plasmid or two singly-resistant plasmids?

The students eventually decide that the drug resistance is due to a single plasmid. They purify plasmid DNA and subject it to restriction endonuclease digestion. Electrophoretic analysis of the digests gives the following fragment sizes:

*Xba*I:	10 kb
*Eco*RI:	5 kb
*Hin*dIII:	1 kb + 9 kb
*Eco*RI + *Xba*I:	2 kb + 3 kb + 5 kb

c) What can you deduce from these results? To what extent is it possible to draw a restriction map with these data?

In the final phase of the investigation, one of the students added DNA ligase and ATP to the *Hin*dIII digest. When this mixture was used to transform *E. coli*, the majority of transformant colonies were ampicillin resistant, while a small proportion were resistant to both ampicillin and chloramphenicol.

d) Suggest possible explanations for this result. What simple experiment could you do to test your hypothesis?

7. PCR

You have identified a protein of interest in your experimental system and have then found the DNA sequence encoding it in an online database (Figure 3). You would now like to clone the sequence for further studies, so decide to use PCR to amplify the 903 bp open reading frame (ORF) from a genomic DNA template.

You first need to design suitable primers to amplify the entire ORF. You consider a few different options for primers of various lengths, as indicated by the lines in Figure 3 (Primers F1-3, R1-3).

Figure 3: Nucleotide sequence to be amplified by PCR.

Six possible primers are shown in Figure 3: 3 Forward and 3 Reverse primers.

a) Which pair of primers would be the optimal primers to choose to amplify the ORF? Briefly explain your reasoning.

b) Write down the nucleotide sequence of each of the two primers you choose (5' to 3').

You need to choose suitable reaction conditions for your PCR.

c) How do you decide what the annealing temperature should be?

You decide to set up three PCR reactions (+DNA template), each with a different annealing temperature: 45 °C, 55 °C and 65 °C. You also set up a control PCR reaction that has no DNA template.

I.e. Four PCR reactions:

PCR Reaction	Annealing Temperature	DNA template?
1	45 °C	Yes
2	55 °Ç	Yes
3	65 °C	Yes
4	55 °C	No

Table 5: PCR reactions set up

Once all of the components have been mixed the PCR reactions are performed as follows: 94 °C for 1 minute and then 30 cycles of 94 °C (30 sec) → X °C (30 sec) → 72 °C (90 sec). Where X is the specific annealing temperature.

At the end of the PCR reaction you prepare the samples for loading on an agarose gel by adding a small volume of 6x DNA sample buffer (0.03% (w/v) Bromophenol Blue, 60% (v/v) Glycerol) to each.

d) Explain the importance of each of the components of the DNA sample buffer.

Once your samples are ready, you want to load them on the gel. However, you realise you've forgotten to label your PCR reactions. You decide to run the gel anyway and hope for the best. The gel is shown in Figure 4. The PCR reactions are loaded in lanes 1-4, alongside a DNA ladder (M).

(TURN OVER for continuation of Question 7)

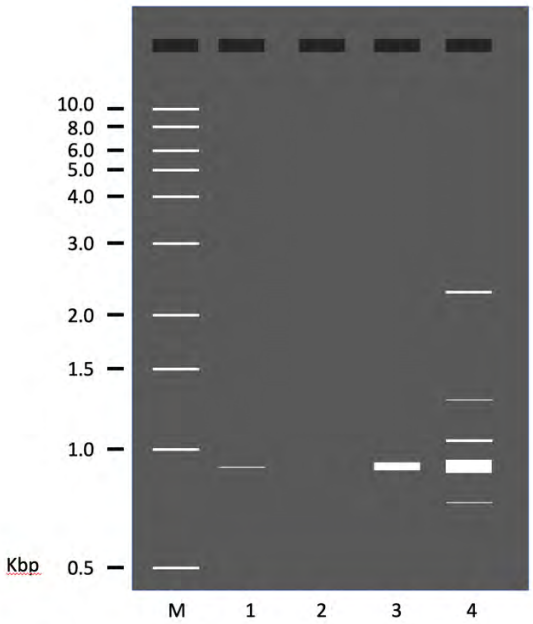

Figure 4: Agarose gel of PCR reactions.

e) How are the DNA bands visualised on the gel?

f) Which lanes of the gel correspond to each PCR reaction? Explain your reasoning.

g) Based on these data, what annealing temperature appears to be *optimal* for this PCR reaction?

h) What is the approximate molecular weight of the protein encoded by the ORF in Figure 3? *Assume the average molecular weight of an amino acid is 110 Da.*

8. Imaging Cell Division

Phase contrast and fluorescence microscopy can be used to image cells.

a) What are the advantages of using phase contrast microscopy? What are the limitations?

b) Some fly lines have genetic mutations that perturb the ability for cells to correctly segregate their chromosomes. Sketch what you might expect to see when looking at their round spermatid cells using phase contrast microscopy?

c) Newt cells can be filmed going through mitosis using phase contrast microscopy. What are the different stages of mitosis that can be clearly seen?

d) A Drosophila syncytial embryo has been stained for DNA and with antibodies against alpha-tubulin and centrosomes. How is it possible to distinguish these different internal structures using fluorescence microscopy?

9. The embryological zoo

Figure 5 shows images of different stages of embryonic development in different species.

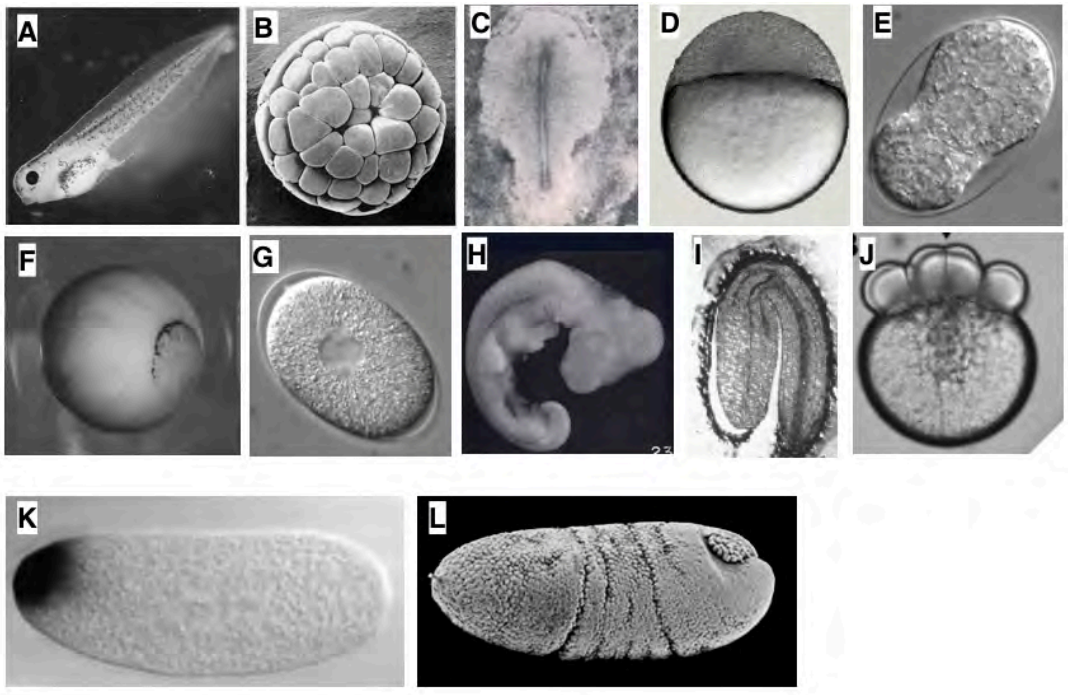

Figure 5. Stages of embryonic development in different species.

a) Panels A-J show representative images of stages of embryonic development from various organisms. Name the organism depicted in each panel (common or Latin names acceptable).

b) When considering panels A-J, which image shows the earliest stage in the development of the organism? Which shows the latest?

c) Panels K-L show representative images of *Drosophila melanogaste*r embryonic development. Suggest a likely identity for the gene product marked by the dark stain at the anterior of the embryo, showed in panel K.

d) Panel L depicts a wild type embryo. What are two ways that panel L would look different if the material stained in panel K was mutated to be non-functional in that embryo?

e) Give two advantages of *Caenorhabditis elegans* as a model organism for the study of development.

f) Give one advantage of *Danio rerio* as a model organism for the study of development.

END OF PAPER