Examination Candidate Number: __________

Desk Number: __________

BSc Degree Examinations 2018-9

Department:
BIOLOGY

Title of Exam:
Molecular Machines

Time Allowed:
2 hours

Marking Scheme:
Total marks available for this paper: 100
The marks available for each question are indicated on the paper

Instructions:

Section A: Answer all questions in the spaces provided on the examination paper
Section B: Answer either question A or question B. Write your answer on the separate paper provided and attach it to the back of the question paper using the cable tie provided.

Materials Supplied:
CALCULATOR
GREEN ANSWER BOOKLET

For marker use only:

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DO NOT WRITE ON THIS BOOKLET BEFORE THE EXAM BEGINS
DO NOT TURN OVER THIS PAGE UNTIL INSTRUCTED TO DO SO BY AN INVIGILATOR
SECTION A: Short Answer / Problem / Experimental Design questions

Answer all questions in the spaces provided

Mark total for this section: 50

1. (a) How can potential energy be stored in the structure of a motor protein?  
(3 marks)

1. (b) Describe how potential energy can be stored in a DNA structure using a specific example.  
(3 marks)

2. A Brownian ratchet model has been proposed to explain how Escherichia coli RNA polymerase couples RNA synthesis to directed motion. Briefly describe the roles of the following in this model and how they might contribute to unidirectional motion of the RNA polymerase.

(a) trigger loop and incoming ribonucleotide triphosphate  
(3 marks)
(b) bridge helix

3. Monomeric helicases contain two RecA-like domains with three conserved sequence motifs listed below. Name the three motifs and state the mechanistic role of the key amino acid residues. (6 marks)

(a) GxxxGK(S/T) in domain 1

(b) DExx in domain 1

(c) Arginine in domain 2

The space above the line should be sufficient for your answer.
4. What is the mechanistic difference between a helicase that unwinds double-stranded DNA passively and one that unwinds actively? (4 marks)

5. Which helicase (passive or active) would display the larger mechanical force-dependence in a single-molecule optical tweezers-based DNA unwinding assay? Provide a brief explanation. (2 marks)

6. The bacterial ribosome has been shown to move in a discontinuous, yet processive manner. The displacement versus time trace below shows one complete mechanical step recorded for a single bacterial ribosome (applied force = 20 pN) as it unwinds an RNA hairpin structure.
(a) Label the pause and translocation phases on the trace.  

(b) Estimate the linear velocity (nucleotides/second) of the ribosome from this data assuming 0.5 nm/nucleotide in single-stranded RNA.

7. What two important roles does elongation factor Tu (EF-Tu) have in peptide bond formation as catalysed by the ribosome?

(a)  

(b)  

8. Briefly describe the following characteristics for myosin II and myosin V noting how mechanistic details in the single-molecule data (e.g. stall force and step size) might distinguish these two motors.

(a) processivity  

(b) stall force  

(c) step size
(d) force-dependence of linear velocity  

(2 marks)

9. Cryogenic electron microscopy (cryo-EM) and X-ray crystallography are used to determine the structural characteristics of the $F_oF_1$ ATP synthase.

(a) Compare the structural characteristics of the $F_oF_1$ ATP synthase as determined by cryo-EM with those obtained from X-ray crystallography, indicating appropriate length scales.  

(4 marks)

(b) Explain how X-ray crystallographic data collected from the $\alpha_3\beta_3$ domain of $F_1$ supports the binding change hypothesis.  

(2 marks)

The space above the line should be sufficient for your answer.
(c) Total internal reflection fluorescence (TIRF) microscopy is used to visualize rotation of γ subunits embedded within immobilized F₁ domains. Each γ subunit is labelled with a biotinylated fluorescent actin filament via a streptavidin linker.

i. Briefly describe how the F₁ domain may be immobilized onto a glass microscope slide in order for rotation to be visualized by TIRF microscopy.

(1 mark)

ii. In the presence of high concentrations of MgATP, the tip of the actin filament is observed to rotate counterclockwise with a tangential velocity of 31.4 μm s⁻¹. If the actin filament is 1 μm long, calculate the number of revolutions per second performed by the γ subunit.

(2 marks)

iii. Explain why the peripheral stalk is likely to counter the tendency of α₃β₃ in the F₁ domain to follow rotation of the γ subunit.

(1 mark)

The space above the line should be sufficient for your answer.
SECTION B: Essay question

Answer one question on the separate paper provided

Remember to write your candidate number at the top of the page and indicate whether you have answered question A or B

Mark total for this section: 50

EITHER

A. Discuss how experiments using optical tweezers and fluorescence microscopy techniques have informed about the directed movement, force generation, processivity and stoichiometry of DNA-dependent molecular machines.

OR

B. Discuss the sequence motifs and organisation of structural domains in myosin and kinesin motor proteins, which provide clues about the function and active form of the motor protein.