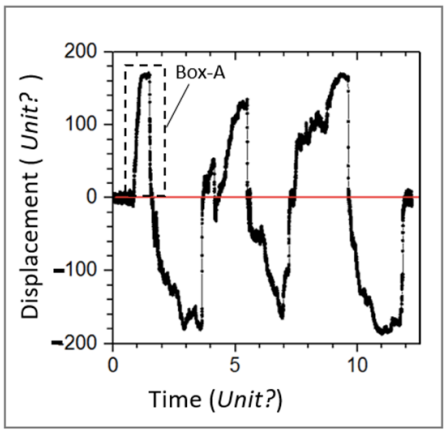
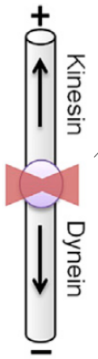
BB101 Endsem, 14 June 2023. MODEL ANSWERS.
EACH QUESTION HAS 9 MARKS. TOTAL MARKS = 45

Q1) Optical Trap data is shown for a Cargo that was extracted from a Cell and then held over a Microtubule.

1) Based on numbers provided along the X and Y axes, write the unit of measurement
2) If we assume that data in Box-A corresponds to a single Kinesin motor, then what is the approximate spring constant (*K*) of this Optical trap?
3) How is this data is different from an experiment that uses a cargo coated with purified kinesin? What do the Negative numbers on Y axis imply?
4) Sketch the data shown inside Box-A on your answer sheet. Explain using this sketch how you will obtain the FORCE-VELOCITY curve from this data.





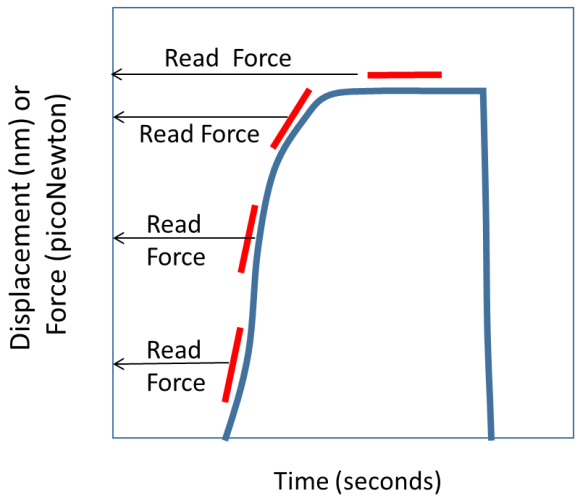
Cartoon that may be drawn to explain the experiment by students. Not included in Qpaper

1) 1-mark. X-axis :- Time (Seconds). Y-axis :- Displacement (Nanometers).

2) 2-marks. A single Kinesin motor generates F ~6 picoNewton force. The displacement (X) is ~170 nanometre. F = K * X, so K = (6 / 170) = 0.035 picoNewton / Nanometre.
There will be some variation about this value, but It should be close to the above value.

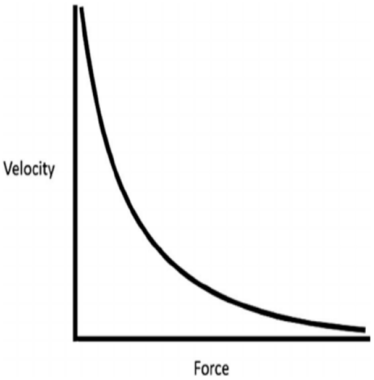
3) 3 marks The negative numbers on Y axis indicate motion towards the minus end of the microtubule. This experiment uses a cargo purified from the cell, so both dynein and kinesin motors can be present on the cargo. That is why the cargo is moving in both directions (towards the plus-end of microtubule and also towards the minus end of microtubule). See the cartoon that has been added to the image above to explain the experiment.

4) 3 marks Force – Velocity Curve :-



Find the slope at different points along Y axis (shown by Red lines). This slope is the velocity of motor at that point.

Read the value of force from the Y axis. So, now you have the velocity at different values of Force. You can plot to obtain the Force-Velocity Curve. Velocity decreases as Force (or Load) increases

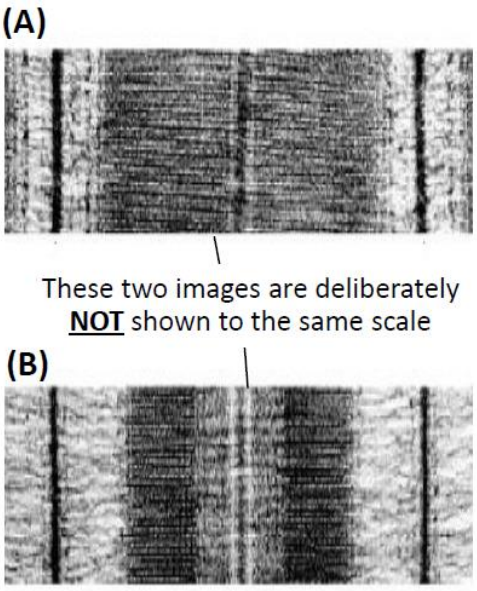


Q2) An Electron Microscope image of a single sarcomere is shown for two states (A) & (B). Which state of the muscle is Relaxed and which state is Contracted? Explain with a labelled sketch.

If F_{MUSCLE} means Force generated by muscle, then explain the meaning of each term in below equation.

$$F_{MUSCLE} = F_{MYO} * (T_{ON}/T_{TOT}) * N_A$$

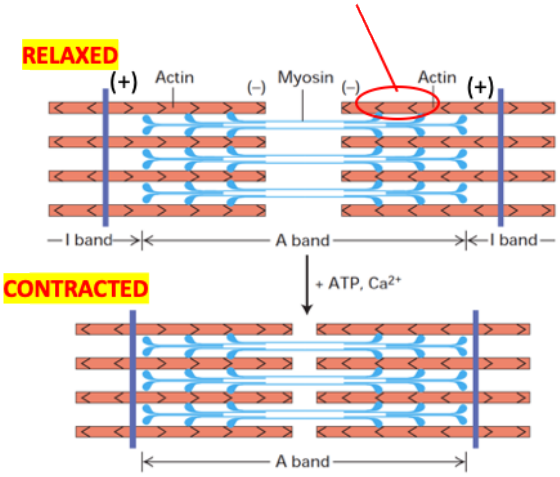
A patient has half the number of myosins in her heart muscles as compared to a normal person. Is her heart likely to be Hypercontractile or Hypocontractile? Explain using the equation.



Which state is Relaxed and which state is Contracted? Explain with a labelled sketch. (3 MARKS)

(A) is Contracted and (B) is Relaxed state. In (A) myosins have pulled the actin filaments together to contract the muscle, so the lighter "Myosin-only region" at the centre of the Z-disk is not visible in (A). The lighter Myosin-only region is clearly visible in (B) because the muscle has relaxed.

The sketch should be similar to what we discussed in class →



Explain the meaning of each term in below equation (3 MARKS)

F_{MUSCLE} is the total force generated by muscle tissue. F_{MYOSIN} is the molecular force from each myosin motor in the sarcomere.

T_{ON} is the time for which myosin is attached to actin. T_{TOT} is the total time for one catalytic cycle of myosin where it hydrolyses 1 ATP ($T_{TOT} = T_{ON} + T_{OFF}$). ($T_{ON}/T_{TOT}$) is the Duty ratio of myosin.

N_A is the available number of myosins that can actually bind to actin. This can be changed by having myosin in ON or OFF state, or if a protein like Tropomyosin is blocking the myosin-binding site of actin

A patient has half the number of myosins in her heart muscles as compared to a normal person. Is her heart likely to be Hypercontractile or Hypocontractile? Explain using the equation. (3 MARKS)

We have only been told that the number of myosins is halved. So, we assume that the force per myosin (=F_{MYOSIN}) and Duty ratio are unchanged. If the overall number of myosins is halved, then the number of available myosins (N_A) that can generate force will also be halved. This muscle will generate less force, so she will have a **Hypocontractile heart**.

Q3) RNA Polymerase (RNAP) walks along DNA to make mRNA. The mRNA of *E. Coli* has an average length of 370 nanometers. You found a new bacteria called *E. Naveen* in which the average mRNA length is only 30 nanometers. What does this tell you about the RNAP of *E. Naveen* ?

Explain using a drawing how you will set up an Optical Trap Experiment to study the RNAP from *E. Naveen*. Draw and explain what the Optical trap data is expected to look like in your experiment.

What does this tell you about the RNAP of *E. Naveen* ? (3 marks)

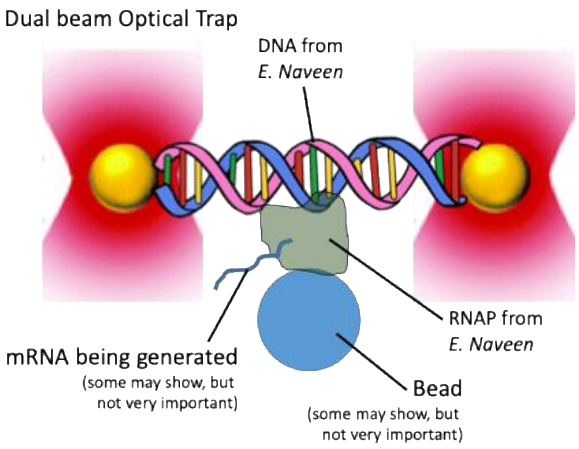
This tells us that the RNAP of *E. Naveen* falls off the DNA much faster than the RNAP of *E.Coli*. So, RNAP from E. Naveen is less "processive" when compared to RNAP from *E.Coli.* E. Naveen-RNAP is likely to have a lower duty ratio. It can only generate force and move along the mRNA in short bursts, therefore it makes short pieces of mRNA.

The difference between E. Naveen-RNAP and E.Coli-RNAP reminds us of the difference between Kinesin (high duty ratio ~ 1) and muscle myosin (low duty ratio ~ 0.2).

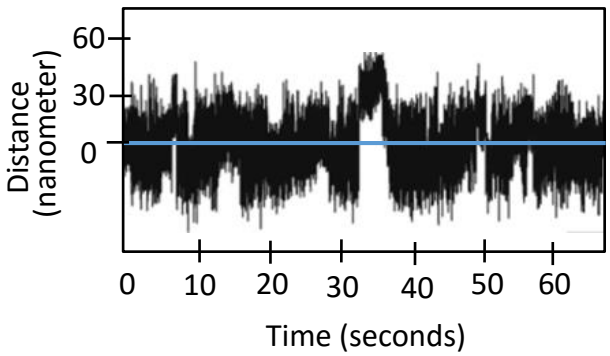
Explain using a drawing how you will set up an Optical Trap Experiment to study the RNAP from *E. Naveen*. Also draw what the expected data will look like (6 marks).

Because E. Naveen-RNAP generates force for a very short time, we will have to use an Dual beam optical trap similar to muscle myosin experiments. The experimental schematic is shown below. The expected data is also shown.

Experimental Setup of Optical Trap



Expected data from Optical Trap



Q4) Each statement below has ONE mistake. Write the corrected statement on your answer sheet.

1 Mark per correct answer (Total 9 marks)

1) *Rotary motion of cilia requires a 9+2 Microtubule arrangement*

Rotary motion of cilia requires a 9+0 Microtubule arrangement
OR
Whip-like/Bending motion of cilia requires a 9+2 Microtubule arrangement

2) *ATP hydrolysis provides energy for Actin depolymerization*
ATP hydrolysis provides energy for Actin polymerization

3) *The plus ends of microtubules usually have a GDP Cap*
The plus ends of microtubules usually have a GTP Cap

4) *Myosin generates the force needed for whip-like motion of Cilia*
Dynein generates the force needed for whip-like motion of Cilia

5) *The anti-cancer drug Taxol increases cell division by binding to Microtubules*
The anti-cancer drug Taxol blocks/reduces/decreases cell division by binding to Microtubules

6) *Emission spectrum of acceptor must overlap with Emission spectrum of donor in a FRET experiment*
Excitation spectrum of acceptor must overlap with Emission spectrum of donor in a FRET experiment

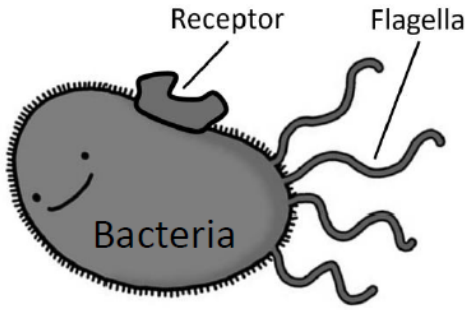
7) *Catenanes work as a Molecular shuttle when the pH is changed*
Rotaxanes work as a Molecular shuttle when the pH is changed

8) *Mitochondria is not found in the Power muscles of the Fly wing*
Sarcoplasmic Reticulum is not found in the Power muscles of the Fly wing

9) *Cystic fibrosis is caused by paralyzed flagella in the lung airway*
Cystic fibrosis is caused by paralyzed Cilia in the lung airway

Q5) What do you understand by Spatial and Temporal sensing in bacteria? Which kind of sensing would require the bacteria to have a memory? Justify your answer.

The bacteria shown here has a single receptor. Which kind of sensing is this bacteria likely to use? Justify your answer.



What do you understand by Spatial and Temporal sensing in bacteria? Which kind of sensing would require the bacteria to have a memory and Why? (5 Marks)

A Bacteria must move around to swim towards attractants (e.g. food) and to swim away from repellants. Therefore, it must sense its surroundings using receptors.

Spatial sensing :- The bacteria compares food at two different spatial locations on its surface at the same point of time. These two locations should be as far away from each other as possible (e.g. at the two ends of the bacteria). It moves towards the side where it senses more food.

Temporal sensing :- The bacteria compares the amount of food at two different time-points while it is moving. This means that the bacteria remembered the amount of food it sensed some time ago, and was able to compare with what it is sensing now. If it senses an increase in the amount of food, then it continues to move in same direction. Temporal sensing therefore requires the bacteria to have some form of memory.

Which kind of sensing is this bacteria likely to use? Justify your answer (4 marks)

This bacteria has only one sensor, so it is not possible for it to compare food at two different spatial locations at the same time. Therefore, this bacteria is likely to use Temporal sensing using the single receptor that it has.