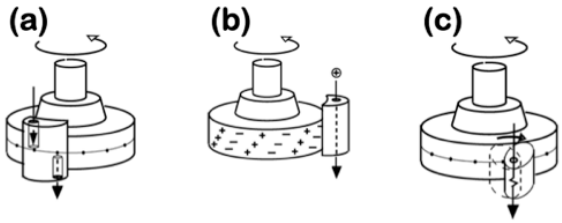
BIO-372 "MICROBIOLOGY" EXERCISES (WEEK 7)

Your Name : _____ Grade : _____

Your Partner: _____ Grade : _____

EXERCISE 1 "BACTERIAL MOTILITY AND CHEMOTAXIS" :

Three different models have been proposed to explain how the bacterial rotary motor works: the *proton turbine* model, the *thermal ratchet* model, and the *mechanochemical* model.



1. Which diagram represents the *proton turbine* model: ☐ (a) ☒ (b) ☐ (c).
Explain how it works:

The surface of the motor rotor has alternating stripes of negative charges and positive charges. These stripes are tilted relative to the long axis of the motor. As a proton travels through the stator complex (from outside the cell to inside the cell) it interacts successively with the stripes of negative charge on the rotor to "pull" the rotor in the direction shown (counter-clockwise). The stripes of positive charge prevent the rotor from turning in the wrong direction, as the proton cannot "back up" in the stator channel.

2. Which diagram represents the *thermal ratchet* model: ☒ (a) ☐ (b) ☐ (c).
Explain how it works:

The stator complex comprises a "half-channel" through which a proton may pass from outside the cell to inside the cell. When the proton enters the upper half-channel, the channel may turn randomly in the clockwise or counter-clockwise directions, driven by random thermal fluctuations. However, only when the upper half-channel turns in the direction of the circular arrow (counter-clockwise) can the upper half-channel and lower half-channel line up to allow passage of the proton into the cell cytoplasm. This model is a thermal ratchet: the energy released by transport of protons down their electrochemical gradient does not create torque *per se*, rather, it somehow "saves" thermal fluctuations in one direction (counter-clockwise) while preventing the rotor from turning in the wrong direction (clockwise).

3. Which diagram represents the *mechanochemical* model: ☐ (a) ☐ (b) ☒ (c).
Explain how it works:

As protons pass through the stator channel they transiently bind to conserved aspartate residues within the channel, thereby neutralizing their negative charge. This interaction causes a conformational change in the stator, which changes its interaction with the rotor, thereby driving the rotor forward (counter-clockwise) by one step. The unbinding of the protons and their release into the cytoplasm causes the stator to return to its original conformation, which changes its interaction with the rotor, thereby driving the rotor forward (counter-clockwise) by another step. This cycle of conformational changes, triggered by binding and unbinding of protons to the stator, drives the rotor forward, step by step.

4. In all three models the source of power is:

☐ A. ATP (adenosine triphosphate) hydrolysis
☐ B. PEP (phosphoenolpyruvate) hydrolysis

Your Name : _____ Grade : _____

Your Partner: _____ Grade : _____

☒ C. PMF (proton motive force)
☐ D. EMF (electron motive force)
☐ E. (A) and (C)
☐ F. (B) and (D)

5. Imagine a bacterial flagellar motor that rotates at 100 Hz with a torque of 10^{-18} joules and a motor mass of 10^{-4} fg (1 femtogram = 10^{-15} grams). What is the motor's power output per kg? Show your work:

rotation frequency: 100 Hz = 100 s^{-1}
angular velocity = 2π * rotation frequency = 6.28 * 100 s^{-1} = 628 * s^{-1}
torque: 10^{-18} joules = 10^{-18} kg * m^2 * s^{-2}
mass: 10^{-4} fg = 10^{-22} kg

power = angular velocity * torque = 628 s^{-1} * (10^{-18} kg * m^2 * s^{-2}) = 6.28 * 10^{-16} kg * m^2 * s^{-3} = 6.28 * 10^{-16} watts

thus

specific power output = power * (motor mass)$^{-1}$ = (6.28 * 10^{-16} watts) * (10^{-22} kg)$^{-1}$ = 6.28 * 10^{6} watts * kg^{-1}

BIO-372 "MICROBIOLOGY" EXERCISES (WEEK 7)

Your Name : _____ Grade : _____

Your Partner: _____ Grade : _____

EXERCISE 2 "BACTERIAL MOTILITY AND CHEMOTAXIS" :

1. Write out the derivation of the Reynolds number (*Re*) equation from the terms defining the *inertial forces* (F_i) component and the *viscous forces* (F_V) component.

$F_i = \rho * S * U^2$

$F_V = \eta * S * U * L^{-1}$

$Re = F_i / F_V = (\rho * S * U^2) * (\eta * S * U * L^{-1}) = \rho * U * L * \eta^{-1}$

where:
ρ = fluid density = 10^3 kg * m^{-3} for water
η = fluid dynamic viscosity = 10^{-3} kg * m^{-1} * s^{-1} for water
S = cross-sectional area of object across the flow
L = length of object with the flow
U = velocity

2. Swimmers can use reciprocal motions to move themselves through water if…
☐ A. The Reynolds number is < 10
☒ B. The Reynolds number is > 200,000
☐ C. Both (A) and (B)
☐ D. None of the above
Explain:

The Reynolds number (*Re*) gives the relative importance of inertial forces (numerator) and viscous forces (denominator) in fluid mechanics.

At high *Re* inertial forces dominate and flow is non-reversible. Thus, swimmers can move using reciprocal motions by alternating fast power strokes (high momentum thus large movement) and slow recovery strokes (low momentum thus small movement).

At low *Re* viscous forces dominate, inertial forces are extremely weak, and flow is reversible. Thus, swimmers cannot move using reciprocal motions, even if the velocity of the power strokes and recovery strokes is different, because the forward and backward movements simply return the swimmer to the place where it began.

3. Imagine a microbe swimming at a velocity of 10 μm per second in water with a length of 1 μm and a surface area across the flow of 1 μm^2. What is the Reynolds number (*Re*) of fluid flow around the microbe?
Show your work:

$Re = \rho * U * L * \eta^{-1} = (10^3 \text{ kg} * m^{-3}) * (10^{-5} \text{ m} * s^{-1}) * (10^{-6} \text{ m}) * (10^{-3} \text{ kg} * m^{-1} * s^{-1})^{-1}$
$Re = 10^{-5}$

4. Imagine a fish swimming at a velocity of 10 m per second in water with a length of 1 m and a surface area across the flow of 1 m^2. What is the Reynolds number (*Re*) of fluid flow around the fish?
Show your work:

$Re = \rho * U * L * \eta^{-1} = (10^3 \text{ kg} * m^{-3}) * (10 \text{ m} * s^{-1}) * (1 \text{ m}) * (10^{-3} \text{ kg} * m^{-1} * s^{-1})^{-1}$

Your Name : _____ Grade : _____

Your Partner: _____ Grade : _____

Re = 10^7

5. Select the statement/s that is/are true for microscopic swimmers like microbes and macroscopic swimmers like fish (multiple responses are possible):

☐ A. Inertial forces are more important for microbes than fish
☒ B. Inertial forces are more important for fish than microbes
☐ C. Inertial forces are equally important for microbes and fish
☒ D. Viscous forces are more important for microbes than fish
☐ E. Viscous forces are more important for fish than microbes
☐ F. Viscous forces are equally important for microbes and fish

Explain:

Inertial forces scale as ($\rho * S * U^2$). Since S (surface area across the flow) and U (velocity) both increase with increasing size, inertial forces increase roughly as the power of 4 of the characteristic dimension. Thus, inertial forces increase rapidly with increasing size; or, conversely, inertial forces decrease rapidly with decreasing size.

Viscous forces scale as ($\eta * S * U * L^{-1}$). Since S (surface area across the flow) and U (velocity) and L (length with the flow) all increase with increasing size, viscous forces increase roughly as the power of 2 of the characteristic dimension. Thus, viscous forces tend to increase more slowly than inertial forces with increasing size; or, conversely, viscous forces tend to decrease more slowly than inertial forces with decreasing size.

Because microbes are small (S and L are small) and move slowly in absolute terms (U is low) they live in a world of low *Re* where the behavior of fluids is dominated by viscous forces.

Because fish are large (S and L are large) and move quickly in absolute terms (U is high) they live in a world of high *Re* where the behavior of fluids is dominated by inertial forces.

BIO-372 "MICROBIOLOGY" EXERCISES (WEEK 7)

Your Name : _____ Grade : _____

Your Partner: _____ Grade : _____

EXERCISE 3 "BACTERIAL MOTILITY AND CHEMOTAXIS" :

1. What is "chemotaxis"? Explain.

Chemotaxis is the directed movement of an organism towards an attractive substance or away from a repellant substance.

2. Describe the strategy that *large* eukaryotic cells use for chemotaxis up a gradient of attractant.

Eukaryotic cells are large enough that they can sense gradients of chemicals in space, because the local concentration at one end of the cell is sufficiently higher than the local concentration at the other end of the cell. Thus, they can sense what is the "correct" direction and steer their movement in that direction.

In sum: eukaryotes sense chemical gradients in *space*.

3. Describe the strategy that *small* prokaryotic cells use for chemotaxis up a gradient of attractant.

Most bacterial cells are too small to sense gradients of chemicals in space, because the local concentration at one end of the cell is essentially the same as the local concentration at the other end of the cell. Instead, they alternate straight runs with tumbles that reorient the bacteria in a random direction. As the bacteria swim, they sense the local concentration of a chemical (attractant or repellant) as a function of time. Runs tend to be longer (probability of a tumble decreases) if the bacteria are swimming in a "good" direction: towards an attractant or away from a repellant. Runs tend to be shorter (probability of a tumble increases) if the bacteria are swimming in a "bad" direction: away from an attractant or towards a repellant. Thus, on average, runs in a "good" direction tend to be longer than runs in a "bad" direction. Eventually they get where they "want" to be…

In sum: prokaryotes sense chemical gradients in *time*.

4. A mutant lacking CheA (kinase) would:
☒ Run all the time
☐ Tumble all the time
☐ Run and tumble with increased tumble frequency (shorter runs)
☐ Run and tumble with decreased tumble frequency (longer runs)
☐ Fail to adapt to changes in attractant concentration
Explain:

The default swimming behavior of bacteria is to run continuously in a single direction. The chemotaxis machinery is essentially a "tumble-generating mechanism". In the absence of the CheA kinase, there would be no phosphorylation of CheY. Since CheY functions as a "switch" protein to change the direction of the flagellar motor from counter-clockwise rotation (running) to clockwise rotation (tumbling), the bacteria would swim continuously without tumbling.

5. A mutant lacking both CheR (methyltransferase) and CheB (methylesterase) would:
☐ Run all the time
☐ Tumble all the time

Your Name : _____ Grade : _____

Your Partner: _____ Grade : _____

☐ Run and tumble with increased tumble frequency (shorter runs)
☐ Run and tumble with decreased tumble frequency (longer runs)
☒ Fail to adapt to changes in attractant concentration
Explain:

Adaptation in the chemotaxis system is mediated by reversible methylation of the chemoreceptors (a.k.a. MCPs, methyl-accepting chemotaxis proteins). The CheR methyltransferase adds methyl groups to the MCPs, which tends to favor the CheA kinase "on" state (tumbling). The CheB methylesterase removes methyl groups from the chemoreceptors, which tends to favor the CheA kinase "off" state (running). The activity of CheB is stimulated by phosphorylation by CheA kinase. Thus, CheB acts as a slow negative feedback loop to return the activated MCP-CheA complex to the inactive state (adaptation). In the absence of CheB, activation of the MCP-CheA complex would not be reversible (as long as the concentration of attractant or repellant did not change), and the system would fail to adapt. Thus, if the bacteria (*cheB* mutant) were transferred from a high concentration of attractant to a low concentration of attractant, it would increase its tumbling frequency indefinitely, without adaptation.

Note: actually, If CheR continues to put methyl groups on the MCPs and there is no CheB to remove them, eventually the MCPs would become fully methylated, which would favor the CheA kinase "on" state, which would mean that the bacteria would tumble most of the time, or maybe even all of the time. Sorry for this ambiguity, it only occurred to me while making up this grading rubric!!! It would be more straightforward to predict the behavior of a *cheB cheR* double loss-of-function mutant, which would respond only to the concentration of attractant or repellant in the local environment, moment by moment, without adaptation.