BIOPHYSICS: Biofilms and Diffusion

Files: **Handouts** for Part 1 (biofilms), Part 2 (Diffusion and the Diffusion constant), Part 3 (Diffusion of Antimicrobial Molecules)

Slides for Part 1 (biofilms), Part 2 (Diffusion and the Diffusion Constant), Part 3 (Antibiotic Resistance; Bioprospecting for New Antimicrobials; Diffusion of Antibiotics and Estimating Molecular Weight)

Lesson Plans

Part 1: Biofilms

Part 2: Diffusion & the Diffusion Constant

Part 3: Diffusion – Estimating the Size of Antimicrobial Molecules

Part 3: Extension: Using the Diffusion Constant to Determine Molecular Weight

This module was developed by Professors Vernita Gordon and Alexandra Eusebi, at the University of Texas, Austin, with funding provided by the National Science Foundation, Division of Civil, Mechanical, and Manufacturing Innovation, award number 1727544, to Vernita Gordon.

If you use any part of this module, please send an email describing your experience to Professor Gordon, gordon@chaos.utexas.edu. Please include the approximate number of students taught. Documenting the use and effectiveness of this module will help us to obtain more funding for outreach and education in the future.

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Driving Questions for the lesson: How do bacteria act in the real world, and what factors impact these behaviors?

PART 1 Biofilms	• How do bacteria interact with our immune systems? • How does the ability to create biofilms affect bacteria resistance to antibiotics? • How does natural selection promote adaptation in bacteria?
PART 2 Diffusion & the Diffusion Constant	• What factors affect diffusion of particles in a 3D liquid or biofilm? • How do changes to properties of the liquid or diffusing particle affect the diffusion constant?
PART 3 Diffusion of Antibiotics	• How can we use mathematical models to describe diffusion constants in real world scenarios? • Extension - How can we use diffusion to estimate the size and molecular weight of an unknown molecule?

TEKS for lesson: This lesson was developed as cross curricular and can supplement: Biology, Physics, & Algebra 1

§112.34. Biology

(4) Science concepts. The student knows that cells are the basic structures of all living things with specialized parts that perform specific functions and that viruses are different from cells. The student is expected to:

(B) investigate and explain cellular processes, including homeostasis and transport of molecules;

(7) Science concepts. The student knows evolutionary theory is a scientific explanation for the unity and diversity of life. The student is expected to:

(C) analyze and evaluate how natural selection produces change in populations, not individuals;

(D) analyze and evaluate how the elements of natural selection, including inherited variation, the potential of a population to produce more offspring than can survive, and a finite supply of environmental resources, result in differential reproductive success;

(E) analyze and evaluate the relationship of natural selection to adaptation and to the development of diversity in and among species; and

(10) Science concepts. The student knows that biological systems are composed of multiple levels. The student is expected to:

(A) describe the interactions that occur among systems that perform the functions of regulation, nutrient absorption, reproduction, and defense from injury or illness in animals;

(11) Science concepts. The student knows that biological systems work to achieve and maintain balance. The student is expected to:

(A) summarize the role of microorganisms in both maintaining and disrupting the health of both organisms and ecosystems; and

(B) describe how events and processes that occur during ecological succession can change populations and species diversity.

(12) Science concepts. The student knows that interdependence and interactions occur within an environmental system. The student is expected to:

(B) compare variations and adaptations of organisms in different ecosystems;

§112.39. Physics

(2) Scientific processes. The student uses a systematic approach to answer scientific laboratory and field investigative questions. The student is expected to:

(H) organize, evaluate, and make inferences from data, including the use of tables, charts, and graphs;

(J) express relationships among physical variables quantitatively, including the use of graphs, charts, and equations.

§111.39. Algebra I

(4) Linear functions, equations, and inequalities. The student applies the mathematical process standards to formulate statistical relationships and evaluate their reasonableness based on real-world data. The student is expected to:

(A) calculate, using technology, the correlation coefficient between two quantitative variables and interpret this quantity as a measure of the strength of the linear association;

(C) write, with and without technology, linear functions that provide a reasonable fit to data to estimate solutions and make predictions for real-world problems.

Objective/s- Write objective/s The student should be able to:	**Assessment:** What will you accept as evidence of student progress toward your lesson objective?
PART 1: • Model the interactions that occur between white blood cells and bacteria (as individual bacterial cells and as a biofilm made up of many aggregated bacteria). • Explain the importance of antibiotics. • Adaptation through natural selection can impact antibiotic resistance and likely promoted the development of the ability of bacteria to form biofilms.	Part 1 • Students will have drawn appropriate models for the activity scenarios and articulate the difference between them. Students will explain interactions between bacteria, white blood cells, antibiotics, and biofilms. Students will articulate how the formation of biofilms affects the effectiveness of white blood cells. Students will also articulate their understanding of how bacteria can be antibiotic resistant, and how not taking a full course of antibiotics can be detrimental. • Formative Assessment will be happening as the teachers walk around and ask questions to students/groups as they are working through activity or handout.
PART 2 • Model diffusion as an effect of random motions of many individual particles. • Explain how changing the motions of individual particles impacts diffusion. • Solve an algebraic equation to quantitatively relate diffusion to different parameters that impact particle motion.	PART 2 • Students will be able to qualitatively describe how changes in parameters such as temperature and viscosity affect particle motion and how this leads to changes in diffusion. • Students will calculate the diffusion constant and particle radius for two given sets of parameters. • Students will algebraically manipulate (re-arrange) the equation for the diffusion constant to solve for particle radius, to understand how knowing a particle's diffusion constant can give information about its size.

	• Formative Assessment will be happening as the teachers walk around and ask questions to students/groups as they are working through activity or handout.
PART 3 • Explain the importance of studying antibiotics and how they interact with bacteria. • Explain the rise of antibiotic resistance and the consequent need to find new antimicrobials • Use scientific method to test compounds for antimicrobial activity by measuring active ingredient diffusion. • If the Extension is used, learn to use a multi-step process to fit an algebraic model to data to approximate the size of an unknown antimicrobial molecule	PART 3 • Students will effectively articulate how bacteria and antibiotics interact in real world settings and how this leads to the spread of antibiotic resistance. • Students will effectively articulate how experimental measurements can be used to determine antibiotic efficacy • If the Extension is used, students will be able to determine molecular size by manipulation and use of an algebraic formula, including using this formula to model data • Formative Assessment will be happening as the teachers walk around and ask questions to students/groups as they are working through activity or handout.

LESSON PLAN: BIOPHYSICS: Biofilms and Diffusion

PART 1: Biofilms

Engagement: Estimated Time: 8 minutes

Overview of Activity: Guided discussion on bacteria and what students discuss what they know about it.

Resources Needed: Giant notepad and markers OR whiteboard and dry erase markers.

Safety Considerations: None.

What teacher is doing:	What the student does:	Possible questions to ask students and anticipated student response
The teacher will have students brainstorm "What do we know about bacteria?" The teacher prepares a large page or whiteboard to write students' responses upon. The teacher facilitates students' discussion and invites groups to share their answers. Teacher guides discussion to include how bacteria are spread, how they are eliminated, and their size. Teacher shows slide with two biofilms: artificial joint with green fuzzy film and hot tub water input pipe (half-blocked with biofilm) and asks students if they can guess what they are looking at. Teacher explains the pictures shown are biofilms and discusses the importance of studying biofilms. ***NOTE: Slides are available with pictures of biofilms and brief introductory facts.***	Students share out responses. If desired, or if prompting is needed, this can be done think-pair-share style: Students think about the question on their own, discuss possible answers with a partner, and then share with the class. Students guess what the photos show.	• What do we know about bacteria? - *Can make you sick* - *Unicellular (one cell)* - *Can be a host* - *Can infect a host* - *Prokaryotic* - *Found everywhere* - *Not all bacteria are bad* • How large are bacteria? • *Microscopic* • How do we kill bacteria? - *Soap/ handwashing* - *Antibiotics* - *Heat* - *Chemicals: bleach, hand sanitizer* - *Magic School Bus* • Why do we study bacteria? Biofilm slides: • What do you think these are?

PART 1: Biofilms

Exploration: Estimated Time: 20 min

Overview of Activity: Students will act out several scenarios that mimic real-world scenario that include white blood cells, bacteria, biofilms, and antibiotics.

Resources Needed: Two hula hoops (for white blood cells), 6 pairs of colorful socks or twelve bandanas/badges. An area that can be cleared of furniture is desirable. (Note: Quantity of resources may need to be adjusted depending on class size.)

Safety Considerations: Basic reminder to students to not run and use common sense with moving around the classroom.

What teacher is doing:	What the student does:	Possible questions to ask students and anticipated student response
Teacher explains the rules for the scenarios and randomly draws students to be the white blood cells, the pharmacist/ doctors, and the bacteria. Teacher reminds students to walk, not run, and to be mindful of not standard classroom safety issues.	Students follow teacher's lead. In general, students that are assigned the role of white blood cell will attempt to eliminate bacteria by "catching" them with hula hoop. Bacteria cells will walk around randomly. Doctor will turn the lights on and off when prompted. Students that can create biofilms will group together. Based on the scenario, some students will sit down when they are caught.	
Scenario 1: non-antibiotic-resistant single-cell bacteria Students assigned the role of bacteria (non-biofilm) will walk around randomly. White blood cells will capture bacteria cells (hula hoop). White blood cells can deliberately walk toward bacteria (this mimics what white blood cells do when they chemotax toward bacteria), although bacteria aren't allowed to deliberately move to avoid them. Those that are engulfed by white blood cells will sit on the floor. Teacher prompts doctors to turn on/off lights to indicate administration of antibiotics; all bacteria will fall to the floor.	**Scenario 1:** When lights are turned on/off by doctor, all non-antibiotic resistant bacteria will fall to the floor.	**Scenario 1:** • Why are the bacteria not allowed to run away from the white blood cells? - *In real life they don't run away from white blood cells.* • Why do all the bacteria cells die when antibiotics are given? - *Antibiotics are designed to kill bacteria.*

Scenario 2: non-antibiotic-resistant bacteria that can form a biofilm Students assigned the role of bacteria will walk around randomly. Bacteria cells that can form a biofilm will group together when they bump into each other and stop moving. Students forming biofilms should make tight clusters, not side-by-side chains. Suggest they stand back to back if they are uncomfortable forming a cluster. White blood cells will capture bacteria cells (hula hoop). Those that are engulfed by white blood cells will sit on the floor. Biofilms that are too large to be engulfed (> 2 students) will remain and continue to grow. Teacher prompts doctors to turn on/off lights; all bacteria at the outer edge of biofilm will fall to the floor. Bacteria in center of biofilm will remain.	**Scenario 2:** Similar start to last scenario. Single bacteria can be engulfed. Bacteria cells that can form a biofilm will group together. Biofilms cannot be engulfed if the hula hoop can't fall to the floor around the "bacterium" – this is one reason the cluster needs to be tight. When lights are turned on/off by doctor or pharmacist, all single bacteria (non-biofilm) will fall to the floor. Bacteria on the outer edges of biofilm will fall to floor. Central biofilm bacteria remain.	**Scenario 2:** • What happens once the bacteria group together to form a biofilm? - *White blood cells couldn't destroy them.* • How did the bacteria in the biofilm respond to antibiotics? - *Central bacteria didn't die.*
Scenario 3: antibiotic-resistant and antibiotic-susceptible single cell bacteria Students assigned the role of non-resistant and resistant bacteria will walk around randomly. Antibiotic-resistant bacteria are given a sock to wear on their arm to set them apart. White blood cells will capture bacteria cells (hula hoop). Those that are engulfed by white blood cells will sit on the floor. Teacher prompts doctors to turn on/off lights to indicate administration of antibiotics; all non-antibiotic resistant bacteria will fall to the floor. Antibiotic resistant bacteria will remain. Teacher tells student to "freeze" the simulation and discusses how bacteria reproduce via mitosis; by splitting from one cell into two genetically identical daughter cells. Teacher then calls upon students that have been eliminated to rejoin the scenario as the second daughter cell for the antibiotic-resistant bacteria still standing (giving	**Scenario 3:** Students assigned the role of bacteria will walk around randomly. Antibiotic resistant bacteria have socks on their hands. Those that are engulfed by white blood cells will sit on the floor. When lights are turned on/off by doctor, all antibiotic-susceptible bacteria (without socks) fall to the floor. Antibiotic resistant bacteria (with socks) will reproduce (signified by adding one student with a sock (bacteria) for each one still standing.	**Scenario 3:** • How is this scenario different? - *Bacteria with socks don't die.* • What do you think will happen to the host? - *The bacteria will grow, and the host will get sicker.* • How do the bacteria adapt? - *More and more bacteria are antibiotic resistant.* • How do bacteria reproduce? What will happen to the cells that are antibiotic resistant over time? - *They split in two (mitosis).* - *The cells that resist antibiotics will have more descendants, so the population of bacteria becomes most or all antibiotic-resistant* • Doctors usually prescribe antibiotics for ten days. What happens if you stop taking

each a sock). The simulation then resumes for a few more seconds for students to see how the population has changed. Teacher prompts students to hypothesize what happens if you stop taking antibiotics before the entire course is taken. Teacher prompts students to hypothesize about what happens over time to the host.		them? How is this different from Scenario 1? - *If you don't take the full course of antibiotics you can get sick again.* - *Now, antibiotics won't work as well because the population of bacteria infecting you is antibiotic-resistant.*
Scenario 4: selective advantage of biofilm-forming ability Students assigned the role of bacteria will walk around randomly. Some bacteria have socks to indicate that they can make a biofilm. Bacteria cells that can make a biofilm will do so when they bump into each other. White blood cells will capture bacteria cells (hula hoop). Those that are engulfed by white blood cells will sit on the floor. Biofilms that are too large to be engulfed (> 2 students) will remain and continue to grow. Teacher prompts doctors to turn on/off lights; bacteria at the outer edge of the biofilm will fall to the floor. Bacteria not on the edge of the biofilm will remain. The bacteria on the new outer edge of the biofilm will and then reproduce (add an additional "biofilm capable" student to the simulation for each; with sock). The innermost bacteria of the biofilm will not reproduce. Teacher allows simulation to resume for a few more seconds to allow students to visualize the population change. Teacher prompts students to hypothesize about the long-term results of the system. ***Note: Make sure students don't confuse the "sock" indicator to mean they are antibiotic resistant. In this scenario the sock indicates which bacteria can make biofilms.***	**Scenario 4:** Students assigned the role of bacteria will walk around randomly. Those that have the ability to make a biofilm will have socks on their hands. If such students bump into each other, they will stick together as a group to form a biofilm. Those that are engulfed by white blood cells will sit on the floor. Those that are too large to be engulfed will continue to stay active. When the lights are turned off/on by the doctor, all single bacteria. Bacteria on the outer edge of the biofilm will also fall to floor. Those in the center of the biofilm will remain. Remaining bacteria will reproduce, by adding one student (with sock to indicate biofilm-forming ability).	**Scenario 4:** • How is this scenario different? - *.* • What do you think will eventually happen to the host? • Why are bacteria in the center of the biofilm less likely to die, even if they are not antibiotic-resistant? - *The antibiotics can't reach them.* • Why are the bacteria in the innermost center of the biofilm less likely to reproduce? - *The oxygen and food they need to grow can't get to them easily.*

PART 1: Biofilms

Explanation: Estimated Time: 20 min

Overview of Activity: Students will draw models of the different scenarios and discuss the similarities and differences between them.

Resources Needed: Guided notes page for biofilm scenario model, or poster paper and markers.

Safety Considerations: None.

What teacher is doing:	**What the student does:**	**Possible questions to ask students and anticipated student response**
Teacher passes out worksheets and prompts students to fill in models on worksheet. Teacher asks students to summarize each scenario and asks students how they would draw each portion of the individual scenario. Teacher calls on students to create one on the overhead. Additional option would be to separate the class into four groups and have each group create a poster of their scenario, modeling the situation and results. ***NOTE: It may be best to complete the models and discussion in two portions; after scenarios 1 & 2, and then again after scenarios 3 & 4.***	Students follow teachers lead. Students discuss the different scenarios and discuss the best way to create their models before generating their and generates their own.	Scenario 1: • Can someone summarize what happened in scenario 1? - *White blood cells destroyed bacteria.* • What would be the best way to model this? - *We cross out the ones that are killed, or color them in to indicate they are dead.* Scenario 2: • What happens once the bacteria group together to form a biofilm? How is this different than Scenario 1? - *The white blood cells couldn't kill the biofilm because it was too big.* - *The bacteria in the biofilm continued to live and grow.* Scenario 3: • How is this different than Scenarios 1 and 2? - *The antibiotic-resistant bacteria didn't die when the lights came on.* - *The antibiotic-resistant bacteria reproduced, so the population of bacteria became more antibiotic-resistant.* Scenario 4: • How is this different than Scenarios 1, 2, and 3? - *The bacteria that could make a biofilm continued to reproduce, so there was a shift in the population's biofilm-forming ability.*

PART 1: Biofilms

Evaluation **Overview of Activity:**		
Resources Needed: Giant notepad and markers OR whiteboard and dry erase markers. **Safety Considerations:** None.		
What teacher is doing:	**What the student does:**	**Possible questions to ask students and anticipated student response**
Throughout the lesson and activity, teacher uses formative assessments to determine how much students are understanding and if they are grasping the new concepts. Summative assessment is completed by evaluating the student work products (generated models and the response on the guided notes/handouts), as well as the post-activity discussion.		

PART 1: Biofilms

Elaboration: Estimated time: 5 minutes **Overview of Activity: Whole class discussion**		
Resources Needed: Guided notes page for biofilm scenario model, or poster paper notepad and markers. **Safety Considerations:** Basic reminder to students to not run and use common sense with moving around the classroom.		
What teacher is doing:	**What the student does:**	**Possible questions to ask students and anticipated student response**
Teacher leads discussion on where we see biofilms in the real world, and the importance of studying them. Teacher asks students to brainstorm questions they have about biofilms and discusses ways these questions could be answered.	Students actively participate in discussion.	• Where else do we see biofilms in real life? - *Teeth* - *Pond surface* - *Mucus that has been expelled (coughing)* • Now that you have thought about how bacteria behave, what questions do you think scientists have when studying biofilms? What kinds of experiments could they preform to investigate these issues? • How do you think understanding the chemical or physical properties of biofilms could help us? • Do you think all biofilms have the same thickness? • How might biofilms differ based on location? For example, in a hot tub drainpipe *vs*. on your teeth?

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PART 2: Diffusion & the Diffusion Constant

Engagement: Estimated Time: 8 minutes
Overview of Activity: The students discuss what they know about how molecules or very small particles move in a fluid.

Resources Needed: Giant notepad and markers OR whiteboard and dry erase markers.
Safety Considerations: None.

What teacher is doing:	What the student does:	Anticipated student response
Guided discussion: • Why do you think bacteria on the outside of the biofilm are killed by antibiotics but not those on the inside? • Why do you think bacteria on the outside of the biofilm reproduce but not those on the inside? Teacher writes responses on whiteboard or notepad, brainstorming-style. Follow-up questions: • How do molecules move in water or another fluid? Teacher shows video: Brownian Motion of Particles (several are available on YouTube, including https://www.youtube.com/watch?v=Xscn-QSmFo4 and https://www.youtube.com/watch?v=f4mp4TWYfBs) Follow-up questions: • What factors do you think influence their movement? • Do you think they will move at the same speed if they are in honey or if they are in air? ***NOTE: If this is separated from Part 1 (Biofilms) to be done on its own, the first question can be skipped and the one above asked without reference to biofilms.***	Students share out responses. If desired, or if prompting is needed, this can be done think-pair-share style: Students think about the question on their own, discuss possible answers with a partner, and then share with the class. Note: This is a common idea, but it is not really important for biofilm growth.	• Initial guided discussion questions: - *Antibiotics have an easier time getting to the outside of the biofilm.* - *Bacteria on the outside of the biofilm have room to grow; bacteria on the inside are too crowded.* - *Bacteria on the outside of the biofilm have an easier time getting oxygen and whatever they need to grow.* • How do molecules move in water or another fluid? - *Randomly* - *Slowly* - *They jitter around* • What factors do you think influence their movement? - *The temperature of the fluid.*

		- *How many particles there are.* - *The size of the container.* • Do you think they will move at the same speed if they are in honey or if they are in air? - *They would move slower in honey because it's thicker.*

PART 2: Diffusion & the Diffusion Constant

Exploration: Estimated Time: 20 min
Overview of Activity: Students will act out diffusion to demonstrate transport of molecules.

Resources Needed: None, other than a classroom or another area. An area that can be cleared of furniture is preferable.
Safety Considerations: Basic reminder to students to not run and use common sense with moving around the classroom.

What teacher is doing:	What the student does:	Possible questions to ask students and anticipated student response
Teacher explains the rules for the scenarios. Teacher reminds students to walk, not run, and to be mindful of not standard classroom safety issues.	Students follow teacher's lead.	
Scenario 1: Diffusion Students act like diffusing molecules moving about with Brownian motion. The scenario begins with all students tightly clustered in one corner of the room, or another well-defined area (masking tape could be used to mark off a region of the floor). Upon the "go" signal, given by the teacher, students begin to wander in random directions, changing directions after every 1-2 steps. If a student runs into a wall or another student, they "bounce" off and go in another direction. The teacher says "stop" when students are distributed throughout the room. Remind students that their movements are random and not purposeful, like on the Brownian Motion video.	**Scenario 1:** When the teacher says "stop" the students stop moving. *NOTE: This seems to be common misconception. While this would cause particles to spread out, it would no longer be Brownian movement of particles or diffusion.*	**Scenario 1:** • What happened? - *We started off all bunched up and then we spread out.* • Why did you all spread out? Were you moving with a plan to spread out? - *No, we were moving randomly with no plan.* • How likely is it that you'd wind up clustered together again? - *Not very likely at all, although it could happen if we waited a very very long time.* - *"It could happen once in the lifetime of the universe"* • What do you think could change how quickly you spread out? - *We could walk faster.* - *We could walk slower.* - *We could walk away from each other on purpose.* • What are ways to change your walking speed? - *Change step size.* - *Change how often you take steps.*

<table>
<tr>
<td>Scenario 2: Diffusion at a different speed
Students act like diffusing molecules, with their walking changed in one of the ways suggested in Scenario 1 (bigger or smaller steps; stepping more, or less, frequently). All students should change their steps in the same way, because they are being a population of <u>identical</u> molecules.

When the students are distributed throughout the room, the teacher says “stop.” If students are moving very slowly and not yet distributed throughout the room, the teacher can wait about the same amount of time as in the first scenario and say “stop.”

If desired, this can be repeated with another way of changing walks.</td>
<td>Scenario 2: When the teacher says “stop” the students stop moving.</td>
<td>Scenario 2:
What happened?
- We spread out more quickly/slowly.
Why?
- Each of us was moving more quickly/slowly.</td>
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PART 2: Diffusion & the Diffusion Constant

Explanation: Estimated Time: 20 min

Overview of Activity: Students will use an algebraic equation to understand the relationship between diffusion and different parameters, such as temperature, viscosity of the surrounding fluid, and the size of the diffusing molecule or particle.

Resources Needed: Handout for the diffusion constant, or poster paper and markers.

Safety Considerations: None.

What teacher is doing:	What the student does:	Possible questions to ask students and anticipated student response
Teacher passes out handouts and walks students through a discussion of the different parameters in the diffusion constant; as shown in the first page of the handout. $$D = \frac{k_B T}{6\pi\eta r}$$	Students actively participate in discussion.	• What will increasing temperature do to the diffusion constant? Why? - *Increase it, because molecules move faster when it's hotter* - *Temperature is in the **numerator** of the fraction.* • What will decreasing temperature do to the diffusion constant? - *Decrease it, because molecules move slower.* • What will increasing the viscosity of the surrounding fluid do to the diffusion constant? Why? - *Decrease it, because molecules move slower, because of increased fluid resistance. Viscosity is in the **denominator** of the fraction.* • What will increasing the size of the particle do to the diffusion constant? Why? - *Decrease it, because molecules move slower, because they have more surface area to be dragged on by fluid. Radius is in the **denominator** of the fraction.*
Teacher: Do you recognize any of these variables?	Students point out the variables they know and take guided notes on the ones they are unfamiliar with.	
Teacher explains what each variable represents and gives background on any terms they not familiar with (i.e., k_B, η). • k_B is the Boltzman constant; relates kinetic energy of a particle to temperature. • T is temperature. • η is the viscosity (How resistant to force a fluid is; how "thick" a fluid is.)	Students discuss how the different parameters would affect the diffusion constant.	
Teacher asks students to make an inference about what it means if it has a higher diffusion constant: *Does this mean the particles spread out faster or slower?*	Students discuss what a larger diffusion coefficient means, comparatively.	
Teacher prompts students to qualitatively think about how the parameter effects particle movement (i.e., particles move faster when it's hotter, so the constant would be higher).		
Teacher has students verify their qualitative statements with quantitative statements: *Looking*	Students discuss how their qualitative statements are	

at the equation, if the temperature was twice as high, how would that effect our diffusion constant?	supported by the location of the variables in the equation.	
Teacher asks students to answer questions on the second page of the worksheet (application of the diffusion constant equation), using the first page for reference. This can be done as individual work or in pairs or small groups.	Students work on the second page of the handout individually.	
Teacher asks students to share their answers. This might be best split up into two parts – students first work on and share answers for questions 1 and 2, and then work on and share answers for questions 3 and 4.	Students share answers to these questions with the class.	
If needed, at the whiteboard or notepad, the teacher can walk students through plugging in parameter values (question 3 and 4B) and algebraic manipulation (question 4A). Alternately, a student can come to the front of the room and work through it.	Students work through/take notes on the problems as the teacher walks through this process so they can understand how to approach questions 3 and 4.	
NOTE: Slides are available with the formula for the diffusion constant that outlines each of the parameters that affect it.		

PART 2: Diffusion & the Diffusion Constant

Evaluation **Overview of Activity:**		
Resources Needed: Handouts for the diffusion constant. **Safety Considerations:** None.		
What teacher is doing:	**What the student does:**	**Possible questions to ask students and anticipated student response**
Throughout the lesson and activity, teacher uses formative assessments to determine how much students are understanding and if they are grasping the new concepts. Summative assessment is completed by evaluating the student work products (the response on the guided notes/handouts), as well as the post-activity discussion.		

PART 2: Diffusion & the Diffusion Constant

Elaboration: Estimated time: 5 minutes
Overview of Activity: Whole class discussion

Resources Needed: Handouts for diffusion constant.
Safety Considerations:

What teacher is doing:	**What the student does:**	**Possible questions to ask students and anticipated student response**
Teacher leads discussion on where diffusion is important in biology and in other situations, and the importance of studying diffusion. After student discussion, if these answers haven't come up, suggest the following: - *Water and molecules made by biological cells can diffuse within those cells* - *Oxygen and nutrients can diffuse to cells (as for the bacteria discussed in the start of this lesson) and waste products can diffuse away* - *Antibiotics and other medicine can diffuse into cells and into intercellular spaces where there's no flow to provide active transport* Teacher asks students to brainstorm questions they have about diffusion and discusses ways these questions could be answered.	Students actively participate in discussion.	• Where is diffusion an important way of transporting (changing the location of) molecules? - *biofilms – nutrients for growth and antibiotics for killing bacteria* • Now that you have thought about how diffusions, what questions do you think scientists have when studying diffusion? What kinds of experiments could they preform to investigate these issues? • How do you think diffusion could help us?

PART 3: Diffusion of Antibiotics

Engagement: Estimated Time: 8 minutes

Overview of Activity: Students discuss what they know about antibiotics, antimicrobials, and antibiotic resistant bacteria.

Resources Needed: Giant notepad and markers OR whiteboard and dry erase markers.

Safety Considerations: None.

What teacher is doing:	**What the student does:**	**Possible questions to ask students and anticipated student response**
The teacher will have students brainstorm "What do we know about antibiotics" The teacher prepares a large page or whiteboard to write students' responses upon. The teacher facilitates students' discussion and invites groups to share their answers. Teacher guides discussion to include the development of antibiotic resistance as a large and growing problem, and the need to find new antibiotics. If this lesson is done in combination with the part of Lesson 1 on natural selection and the spread of antibiotic resistance, the teacher may prompt students to remember what happened in Scenario 3. If students are reluctant to share, teacher will ask students to talk to a partner for 2 minutes before a group share out. ***NOTE: Slides on antibiotic resistance are available.***	Students share out responses.	• What do we know about antibiotics? - *Type of medicine* - *Doctor prescribes them* - *Kill bacteria* - *Don't work against viruses* • Do antibiotics always work successfully to kill bacteria and thereby cure disease? • *No – antibiotic resistance* • How could we make this better? - *Try to prevent bacterial illness (handwashing, etc.)* - *Take the full course of antibiotics as prescribed* - *Find new ways to kill or inhibit bacteria, including new antibiotics*

PART 3: Diffusion of Antibiotics

Exploration: Estimated Time: 20 min **Overview of Activity:**		
Resources Needed: **Safety Considerations:**		
What teacher is doing:	**What the student does:**	**Possible questions to ask students and anticipated student response**
Option 1: Hands-On Activity NOTE: Preparation for the exploration is required 24-48 hours prior to the lab, including creating agar plates streaked with bacteria. Labs restricted from using bacteria specimens can use food coloring spread on agar plates as a replacement. Instruction module, including detailed explanation and videos on lab prep and experimentation are provided here: https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1002044 **Option 2: Video presentation of Lab** If it is not possible to complete the lab as a hands-on activity, students can follow along with the video to watch how the work is performed or listen to the teacher's description of the lab and observations they made along the way. Video is available online: https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1002044#sec008		

PART 3: Diffusion of Antibiotics

Explanation: Estimated Time: 20 min

Overview of Activity: Students discuss and answer questions about how inhibition of bacteria relates to diffusion of an antibiotic and therefore the size of the antibiotic molecule.

Resources Needed: Handout for "Diffusion of antibiotics", or poster paper and markers.

Safety Considerations: None.

What teacher is doing:	What the student does:	Possible questions to ask students and anticipated student response
Teacher passes out guided notes/handout and walks through the equation describing how the size of the inhibition zone (L^2) depends on different experimental parameters. $L^2 = 4DT_c\, ln(C_o) + F(D, T_c, C_c)$ Where, L is the radius of the inhibition zone; D is the diffusion constant; T_c is the critical preincubation time, beyond which no inhibition of bacterial growth is seen; C_o is the prepared concentration of active ingredient; C_c is the lowest (critical) concentration of antimicrobial compound that is observed to inhibit bacterial growth. Two key parameters (antibiotic concentration, C_c, and pre-incubation time, T_c) can be varied and the resulting measurement of inhibition zone size can be fit, using a linear function, to find the information needed to determine the antibiotic molecule's diffusion constant. Teacher walks through the similarity of this model to linear equations in slope-intercept form, and how preforming a	Students follow teacher's lead.	• What part of this equation corresponds to the independent variable *x* (which we controllably vary in our experiment) and what part corresponds to the dependent variable *y* (what we measure in our experiment)? - $ln(C_o)$ *is the independent variable. Students may say that* C_o *is the independent variable – this is also true. We use the natural log of* C_o *here because that makes this equation linear.* - L^2 *is the dependent variable.* • What part of this equation corresponds to the slope *m* and what part corresponds to the intercept *b*? - $4DT_c$ *is the slope.* - $F(D, T_c, C_c)$ *is the intercept* • If you know the slope here, what else do you need to know to find the molecule's diffusion constant? - *Critical pre-incubation time* T_c*.* • How could you find out what T_c. is? - *Try different pre-incubation times and see what works*

linear regression will help solve for unknown quantities. Teacher prompts students to follow the examples on the handout and apply their understanding to the "Think about it" on the handout. ***NOTE: Slides are available with a walk-through of the formula describing the size of the inhibition zone, plus information relating diffusion to molecular size.***	Students discuss the "Think about it" questions and answer them on the worksheet. NOTE: This approach won't work because this isn't the type of thing that is already known – it needs to be measured afresh for each organism, antibiotic, and set of growth conditions. This could be a good opportunity to talk about the process of doing science.	- *Look it up in a book/Google, or ask a scientist/doctor/expert.*

PART 3: Diffusion of Antibiotics

Evaluation
Overview of Activity:

Resources Needed: Giant notepad and markers OR whiteboard and dry erase markers.
Safety Considerations: None.

What teacher is doing:	What the student does:	Possible questions to ask students and anticipated student response
Throughout the lesson and activity, teacher uses formative assessments to determine how much students are understanding and if they are grasping the new concepts. Summative assessment is completed by evaluating the student work products (response on the guided notes/handouts), as well as and the post activity discussion.		• Can someone summarize what we did in the lab today? • What data did we collect? • Can someone list several reasons why this type of analysis is beneficial?

PART 3: EXTENSION: Using Diffusion to Estimate the Molecular Weight of Antibiotics

Elaboration: Estimated time: 5 minutes
Overview of Activity: Whole class discussion

Resources Needed: Handout for "extension – using diffusion to estimate the molecular weight of antibiotics", or poster paper notepad and markers.
Safety Considerations: None.

What teacher is doing:	What the student does:	Possible questions to ask students and anticipated student response
Teacher leads discussion on how to find new antibiotics, including looking for things found in nature that have antibiotic properties ("bio-prospecting"). so. ***Note: Slides on Bioprospecting are available as part of this lesson plan.*** Teacher says: A key step of bioprospecting is figuring out what molecule(s) in a natural product are killing or inhibiting bacteria. That knowledge allows the antibiotic molecule(s) to be produced in greater quantity and given in more concentration. Knowing an antibiotic's molecular properties can also help scientists understand <u>how</u> it kills or inhibits bacteria and can give warnings for ways in which it might hurt people or animals. ***NOTE: An handout for this extension is provided ("extension - Using Diffusion to Estimate the Molecular Weight of Antibiotics"). Slides for this extension are***	Students actively participate in discussion.	• How do we find new types of antibiotics? - *Take existing antibiotics and change them* - *Create a lot of different types of molecules and test them all to see which ones kill or inhibit bacteria* - *Use viruses to kill bacteria* - *Look for things found in nature that kill or inhibit bacteria* • If we find something in nature that has antibiotic properties, what do scientists need to do to turn that discovery into a useful medicine? - *Make sure it doesn't hurt people and animals* - *Make sure it works in people and animals* - *Figure out how to give it to people and animals* • If we find something in nature that has antibiotic properties, what do scientists need to do to turn that discovery into a useful medicine? - *Make sure it doesn't hurt people and animals* - *Make sure it works in people and animals* - *Figure out how to give it to people and animals* • How could finding an unknown antibiotic molecule's size, using the type of experiments discussed today, help with the discovery and

included in "Diffusion of Antibiotics and Estimating Molecular Weight."		development of new antibiotics? • Besides size, what are some other molecular properties that scientists might want to know about new antibiotics? - *Molecular structure – there are examples of this in the last page of the handout* - *How does it interact with molecules in human or animal cells?*