HIV:

A Study of Infection, Spread, Treatment, and Drug Development

This unit will help students understand HIV infections in individuals and populations along with drug development and treatment strategies. This will be done through roundtable discussion, case study analysis, cladistics, bacterial transformation, enzyme kinetics, and protein crystallization.
NIH Curriculum: HIV - A Study of Infection, Spread, Treatment, and Drug Development
Author: Jonathan Benskin – Boca Raton Community High School

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The content is solely the responsibility of the author and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health.

Additional information regarding the Bench to Bedside project is available at http://www.cpet.ufl.edu/bench.

Please direct inquiries to the Center for Precollegiate Education and Training at cpet@cpet.ufl.edu.

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## Contents

Author’s Note .............................................................................................................. 6

Introduction .................................................................................................................. 7

Tips about this Curriculum .......................................................................................... 8

Lesson Summaries ........................................................................................................ 10

Lesson Sequencing Guide ............................................................................................ 11

Vocabulary .................................................................................................................... 12

Next Generation Sunshine State Standards – Science .................................................. 15

Advanced Placement Biology Learning Outcomes & Science Practices ....................... 16

Advanced International Certificate of Education (AICE) Biology Learning Outcomes .... 18

Background Information .............................................................................................. 19

LESSON ONE: “HIV? Are you positive?” ...................................................................... 20

Student Pages: HIV Case Study – Student Handout ..................................................... 24

STUDENT PAGES: QUESTIONS FOR HIV CASE STUDY ........................................... 27

TEACHER PAGES: HIV CASE STUDY QUESTIONS - ANSWER KEY ....................... 28

TEACHER PAGES: POWERPOINT / LECTURE OUTLINE ........................................ 31

STUDENT PAGES: QUESTIONS FOR HIV / AIDS TIMELINE .................................... 33

TEACHER PAGES: QUESTIONS FOR HIV / AIDS TIMELINE – ANSWER KEY .......... 34

LESSON TWO – “SO MANY STRAINS, SO LITTLE TIME” ........................................... 35

STUDENT PAGES: THE MAPPING OF HIV / AIDS AROUND THE WORLD – STUDENT HANDOUT ................................................................. 40

TEACHER PAGES: THE MAPPING OF HIV / AIDS AROUND THE WORLD – ANSWER KEY ................................................................. 42

STUDENT PAGES: PHYLOGENETIC TREE CREATING BASED ON SCIENTIFIC LITERATURE – STUDENT HANDOUT ................................. 44

TEACHER PAGES: PHYLOGENETIC TREE CREATING BASED ON SCIENTIFIC LITERATURE – ANSWER KEY ............................ 46

STUDENT PAGES: CASE STUDY: SOUTH AFRICA – STUDENT INSTRUCTIONS .......... 48

FIGURE 1 – PHYLOGENETIC TREE ............................................................................. 49

LESSON THREE: “A MUTANT IN OUR MIDST, PART 1” ........................................... 52

STUDENT PAGES – DRUG DISCOVERY CARDS............................................................ 57

STUDENT PAGES – DRUG DISCOVERY AND DEVELOPMENT – STUDENT HANDOUT: ................................................................. 63

TEACHER PAGES: DRUG DISCOVERY AND DEVELOPMENT Student Handout: Drug Discovery and Development – ANSWER KEY ................................................................. 65

STUDENT PAGES: HIV MEDICATION TYPES – STUDENT HANDOUT ....................... 67

TEACHER PAGES: HIV MEDICATION TYPES – ANSWER KEY ................................. 68

LESSON FOUR: “A MUTANT IN OUR MIDST, PART 2” .............................................. 69

STUDENT PAGES: BACTERIAL TRANSFORMATION – STUDENT HANDOUT ............... 74

TEACHER PAGES: BACTERIAL TRANSFORMATION – ANSWER KEY ......................... 76

LESSON FIVE: “OPTIMIZE AND INHIBIT – AND KEEP YOUR JOB” ....................... 78

STUDENT PAGES: ENZYME OPTIMIZATION – STUDENT INSTRUCTIONS ............... 86

STUDENT PAGES: ENZYME OPTIMIZATION QUESTIONS ......................................... 88
<table>
<thead>
<tr>
<th>Page Reference</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>89</td>
<td>TEACHER PAGES – ENZYME OPTIMIZATION QUESTIONS – ANSWER KEY</td>
</tr>
<tr>
<td>90</td>
<td>STUDENT PAGES: ENZYME INHIBITION – STUDENT INSTRUCTIONS</td>
</tr>
<tr>
<td>92</td>
<td>STUDENT PAGES: ENZYME INHIBITION QUESTION</td>
</tr>
<tr>
<td>94</td>
<td>TEACHER PAGES: ENZYME INHIBITION QUESTIONS – ANSWER KEY</td>
</tr>
<tr>
<td>95</td>
<td>LESSON SIX: “THE PERFECT CRYSTAL”</td>
</tr>
<tr>
<td>100</td>
<td>STUDENT PAGES: PROTEIN CRYSTALLIZATION – STUDENT INSTRUCTIONS</td>
</tr>
<tr>
<td>104</td>
<td>STUDENT PAGES: PROTEIN CRYSTALLIZATION – STUDENT QUESTIONS</td>
</tr>
<tr>
<td>106</td>
<td>TEACHER PAGES: PROTEIN CRYSTALLIZATION – ANSWER KEY</td>
</tr>
</tbody>
</table>
Author’s Note

As a child growing up in the 1980s and 1990s, I remember hearing about the human immunodeficiency virus (HIV) on the news and seeing pictures of the toll it has on the human body. HIV was probably the first global disease that I was aware of. I remember having a certain fear of the disease simply because I did not understand anything about it except for the fact that “you can’t get rid of it, and it will kill you.” Although HIV is still extremely prevalent, it has somewhat faded from the limelight of the media. There are many groups that are active in HIV education for youth, but I believe that the mindset of “you can’t get rid of it, and it will kill you” still exists. I believe that this shows a lack in the understanding of the HIV virus and infection.

Many high school science classes teach some facts or details about HIV, but they are usually limited to general structure and function of a virus. History of the virus, epidemiology, drug development and treatment are usually ignored- possibly from lack of time or educator knowledge. However, I feel that this is the information that should be focused on. I created this unit to promote the student’s understanding of general biology concepts, and also allow them to have a much better understanding of what exactly HIV is, what it does, and what can be done to treat it. It is my hope that after the completion of this unit, students will know much more about this disease than I ever did when I was growing up.
Introduction

After teaching Advanced Placement Biology for the last 6 years, I have come to the understanding that some laboratory activities are not effective for instruction. Students will sit and complete the activity but have no idea how, or if, it actually pertains to their own lives. And in many cases, the activity does not have any real-life application, or even use technologies or methods used in a current university or research setting. I kept all of this in mind while developing this curriculum.

This curriculum was designed to unify many of the recommended lab topics taught in upper-level biology classes. It is my belief that if many of these laboratory techniques can be linked together then it will better facilitate student understanding by allowing them to see the “big picture”. If this entire unit is completed, students will identify a problem (HIV), understand how it spreads and what affect it has on populations, and use current research methods to better understand how it is researched and treated. Current laboratory HIV research methodology includes protein crystallization, enzyme kinetic analysis and enzyme inhibition- all of which are actually completed in this unit.

I wanted to safely and accurately mimic HIV research when developing activities for this unit. After spending 2 weeks in a University of Florida research laboratory, I feel I have successfully created a unit which faithfully replicates current research being done specifically on HIV-1 protease inhibition. The inhibition of this HIV molecule is one the many ways HIV is being researched, but it is arguably one of the most popular. After the completion of this unit, I believe students will not only have a much clearer understanding of HIV, but also understand essential laboratory skills which will actually help them in their secondary education and beyond.
Tips about this Curriculum

Lesson Plan Format: All lessons in this curriculum unit are formatted in the same manner. In each lesson you will find the following components:

KEY QUESTION(S): Identifies key questions the lesson will explore.

OVERALL TIME ESTIMATE: Indicates total amount of time needed for the lesson, including advanced preparation.

LEARNING STYLES: Visual, auditory, and/or kinesthetic.

VOCABULARY: Lists key vocabulary terms used and defined in the lesson. Also collected in master vocabulary list.

LESSON SUMMARY: Provides a 1-2 sentence summary of what the lesson will cover and how this content will be covered. Also collected in one list.

STUDENT LEARNING OBJECTIVES: Focuses on what students will know, feel, or be able to do at the conclusion of the lesson.

STANDARDS: Specific state benchmarks addressed in the lesson. Also collected in one list.

MATERIALS: Items needed to complete the lesson. Number required for different types of grouping formats (Per class, Per group of 3-4 students, Per pair, Per student) is also indicated.

BACKGROUND INFORMATION: Provides accurate, up-to-date information from reliable sources about the lesson topic.

ADVANCE PREPARATION: This section explains what needs to be done to get ready for the lesson.

PROCEDURE WITH TIME ESTIMATES: The procedure details the steps of implementation with suggested time estimates. The times will likely vary depending on the class.

ASSESSMENT SUGGESTIONS: Formative assessment suggestions have been given. Additionally, there is a brief summative assessment (pre/post test) that can be given. Teachers should feel free to create additional formative and summative assessment pieces.

EXTENSIONS: (ACTIVITIES/LITERATURE) There are many activities and reading sources available to augment and enhance the curriculum. They have been included. If you find additional ones that should be added, please let us know.

RESOURCES/REFERENCES: This curriculum is based heavily on primary sources. As resources and references have been used in a lesson, their complete citation is included as well as a web link if available. All references and resources are also collected in one list.

STUDENT PAGES: Worksheets and handouts to be copied and distributed to the students.

TEACHER PAGES: Versions of the student pages with answers or the activity materials for preparation.

Collaborative Learning: The lessons in this curriculum have been developed to include many collaborative learning opportunities. Rather than presenting information in lecture format and teacher driven, the activities involve the students in a more engaged manner. For classrooms not accustomed to using collaborative learning strategies, have patience. It can be difficult to communicate instructions, particularly for students who are
visual learners. For these students, use of visual clues such as flowcharts and graphics can help them understand how they are to move to different groups.

**Groups:** Most of the lessons are carried out in groups. While it isn’t necessary for students to remain in the same groups the entire unit, if they work well together, it may foster students to think deeper as they are comfortable with their teammates and willing to ask questions of each other.

**Inquiry-based:** The lessons in the curriculum invite students to be engaged and ask questions. They work through background information in a guided fashion, but are challenged to think beyond what they have read or done. The teacher serves as the facilitator in these activities, not the deliverer of information.

**Technology:** Lessons have been written to be mindful of varying availability of technology in schools and homes. Some of the lessons would be very well suited to online environments and if your students are able, you might wish to engage in some of the technology modifications.

**Content:** Often we teach in a manner that is very content heavy. With high-stakes testing the norm, students are pushed to memorize and regurgitate numerous isolated facts. There is so much content that must be covered in a biology class, for example, that often it is difficult to synthesize those discrete facts into a compelling context or a story. This unit provides that opportunity: to take concepts learned such as muscles have a lot of glycogen or DNA codes for RNA, and put them in the context of disease. The lessons aren’t designed to teach students what lysosomes do or transcription is, but rather why these ideas are important and how they can be used by researchers.

**Implementation notes:** This curriculum should be modified and adapted to suit the needs of the teacher and students. To help make implementation easier in this first draft, notes have been included in lessons as needed.

**Extensions:**

**Science Subject:** Biology – AP, AICE, IB

**Grade and ability:** 10-12 grade Advanced Placement/AICE/IB Biology.

**Science concepts:** HIV, AIDS, viral transmission, retrovirus, immune system, phylogenetic tree, South Africa, World HIV maps, drug development, HIV-1 Protease, mutations, protein crystallography, x-ray crystallography, scientific presentations of data
Lesson Summaries

LESSON ONE: “HIV? Are you positive?”
This lesson will focus on how HIV will affect the individual's body. First, a modified roundtable activity will be used to discuss what is known about HIV. After this discussion, a case study will illustrate the modes of HIV transmission from human to human followed by a short lesson into how HIV can progress into AIDS.

LESSON TWO: “So Many Strains, So Little Time”
This lesson will focus on the geographic origin of HIV and how it mutated and moved to different parts of the world. Students will read part of a scientific paper to create a phylogenetic tree and use the Internet to construct world maps showing the frequency of different HIV strains around the world. Additionally, students will learn about an exceptionally strange situation in which the South African government denied that HIV was the cause of AIDS and how the world responded.

LESSON THREE: “A Mutant in Our Midst, Part 1”
In “A Mutant in Our Midst, Part 1”, students will learn general information about how medicinal drugs are developed and researched. Students will complete an investigation into what types (groups) of medicines are available to treat HIV/AIDS infections and how they generally work.

LESSON FOUR: “A Mutant in Our Midst, Part 2”
In “A Mutant in Our Midst, Part 2”, students will simulate the first steps of HIV-1 protease research by transforming bacteria with fluorescent proteins and cloning genes. A focus on HIV-1 protease will be used as an example target for medications.

LESSON FIVE: “Optimize and Inhibit – and Keep Your Job”
Students will optimize enzyme kinetics of simulated HIV-1 protease. Additionally, students will work to inhibit an enzymatic reaction (simulated HIV-1 protease) by the manipulation of different inhibitors in order to find the most effective product.

LESSON SIX: “The Perfect Crystal”
Students will understand how, and why, proteins are crystallized using the crystallization of lysozyme as an example protein. Students will then take their data (from lessons 3, 4 and 5) and create a poster that shows what they did in the drug discovery/development process.
Lesson Sequencing Guide

All lessons are based on a 55 minute class session:

<table>
<thead>
<tr>
<th>WEEK 1</th>
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<th>DAY 2</th>
<th>DAY 3</th>
<th>DAY 4</th>
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<td>LESSON 1</td>
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<td></td>
<td>“The Perfect Crystal”</td>
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Vocabulary

AIDS - Acquired immune deficiency syndrome. Caused by HIV and is classified as “AIDS” when CD4+ T cell count is less than 200 cells/mm³.

AIDS denialists - Group of individuals who deny that HIV is the cause of AIDS.

Ampicillin - An antibiotic that is semi-synthetic and is closely related to penicillin.

Antibodies - Proteins that an individual’s immune system makes in order to fight an infectious agent.

Antiretroviral drugs - Drugs intended to fight retroviruses, such as HIV.

Bushmeat - Product of the killing of wild animals (most commonly primates) for subsistence or commercial purposes.

CD 4+ T cells - One of the specific human immune system’s cells, or white blood cell, that HIV infects.

Circulating recombinant forms (CRF) - Forms of HIV that are a combination of the general subtypes.

Competency - A state that a bacterial cell has to be in, in order to accept a plasmid.

Competitive inhibitor - The inhibitor “competes” with the normal substrate for the active site.

Conformation - The proper 3-dimensional shape that a protein takes. This shape will determine its function.

Crystallization - An organized structure of purified molecules which align themselves in repeating units, forming the crystal. A pure protein crystal can then be analyzed using x-ray crystallography to determine the tertiary or 3-D structure of the molecule of interest.

Crystallization - This process allows macromolecules (such as proteins) to form crystals. The macromolecule will be “locked” in place which allows further study.

Denature - When a protein's conformation changes. If this protein is an enzyme, this will usually render it useless.

Divergent evolution – The accumulation of differences in a species based on the physical separation of the species.

Durban Declaration - Article published in Nature that was intended to encourage Thabo Mbeki to condone antiretroviral treatments for those with HIV/AIDS.

Enzyme - A molecule found in biological systems that speeds up a reaction by reducing the activation energy.

HAART - Highly Active AntiRetroviral Therapy. The most commonly used drug combinations to fight HIV/AIDS.
**Hanging drop vapor diffusion method** - Method used to crystallize proteins in which the protein is suspended above a well of solution (see below for methodology).

**Hemoglobin** - Protein that is found in red blood cells and shuttles O₂ and CO₂ around the body.

**High-throughput technology** - Technology that allows high-speed, automated testing of samples.

**HIV- Human immunodeficiency virus** - Retrovirus that is the cause of AIDS.

**Horseradish peroxidase** - A common enzyme that catalyzes a variety of reactions.

**IC₅₀** - The amount/concentration required to inhibit the reaction by 50%

**In vitro** - In test tubes and petri dishes.

**In vivo** - In living organisms.

**Inhibitor** - A molecule that will slow down an enzymatic reaction.

**Kgalema Motlanthe** - President of South Africa from 2008 – 2009; promoted the treatment of HIV with antiviral drugs.

**LD₅₀** - Median lethal dose, or the dose that will kill 50% of the tested population.

**Lead compounds** - The most likely drug candidates that will be investigated.

**Lock and key mechanism** - The first model used to explain how an enzyme and substrate interacted. It was meant to show the action of the substrate entering the enzyme is extremely specific.

**Lymph Nodes** - Nodules in the lymphatic system where some white blood cells are produced, and lymph is filtered.

**Lymphocytes** - White blood cells.

**Molecular entities** - Potential drugs

**Non-competitive inhibitor** - The inhibitor fits into a different site from the active site, but when doing do, changes the normal conformation of the active site, which prohibits the normal substrate from entering.

**Nucleotides** - Fundamental building blocks of DNA (adenine, thymine, guanine, cytosine).

**o-Dianisidine** - A dye that, when oxidized, turns from clear to brown.

**Pandemic** - Worldwide epidemic.

**Polypeptide** - A polymer (chain) of amino acids
**Primary sequence** - The order of amino acids that make up the protein

**Retrovirus** - RNA virus that uses reverse transcriptase to integrate it genetic material into the host’s genetic material.

**Reverse Transcriptase** - The enzyme that retroviruses use to copy and insert their RNA into the host’s DNA.

**Secondary Infections** - Opportunistic infections that are the result of the HIV infection.

**Selective media** - Growth media that will not allow certain bacteria to grow, but will allow others (with a specific trait) to grow.

**Seroconversion** - The development of detectable amounts of HIV antibodies in the blood.

**Sitting drop vapor diffusion method** - Method used to crystallize proteins in which the protein is sitting above a well of solution (see below for methodology).

**SIV** - Simian immunodeficiency virus. Precursor to HIV and carried by apes and monkeys.

**Substrate** - The molecule that the enzyme acts upon.

**Target molecule** - The molecule that is not working properly, or needs to be inhibited. This molecule is the target of the drug in development.

**Thabo Mbeki** - President of South Africa from 1999 –2008; had controversial view of fighting HIV/AIDS with antiretroviral medicine.

**Transformation** - The insertion of a plasmid into bacterial cells.

**Virion** - Virus particle.

**Virions** - Fully-formed viruses that are traveling around the body.

**X-ray crystallography** - The use of x-rays to determine the atomic structure of a crystallized molecule.
Next Generation Sunshine State Standards – Science

<table>
<thead>
<tr>
<th>Benchmark</th>
<th>Lesson</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SC.912.L.14.6:</strong> Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health.</td>
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<tr>
<td><strong>SC.912.L.16.7</strong> Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology.</td>
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<td><strong>SC.912.L.16.10</strong> Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues</td>
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<td><strong>SC.912.L.18.4</strong> Describe the structures of proteins and amino acids. Explain the functions of proteins in living organisms. Identify some reactions that amino acids undergo. Relate the structure and function of enzymes.</td>
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<td><strong>SC.912.L.18.11</strong> Explain the role of enzymes as catalysts that lower the activation energy of biochemical reactions. Identify factors, such as pH and temperature, and their effect on enzyme activity.</td>
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</tbody>
</table>
### Advanced Placement Biology Learning Outcomes & Science Practices

<table>
<thead>
<tr>
<th>Outcomes &amp; Practices</th>
<th>Lesson</th>
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<tbody>
<tr>
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<tr>
<td>LO 1.17</td>
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<tr>
<td>The student is able to pose scientific questions about a group of organisms whose relatedness is described by a phylogenetic tree or cladogram in order to (1) identify shared characteristics, (2) make inferences about the evolutionary history of the group, and (3) identify character data that could extend or improve the phylogenetic tree.</td>
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<td>LO 2.29</td>
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<td>The student can create representations and models to describe immune responses</td>
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<td>LO 3.29</td>
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<td>The student is able to construct an explanation of how viruses introduce genetic variation in host organisms</td>
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<td>LO 4.1</td>
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<td>The student is able to explain the connection between the sequence and the subcomponents of a biological polymer and its properties.</td>
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<tr>
<td>LO 4.2</td>
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<tr>
<td>The student is able to refine representations and models to explain how the subcomponents of a biological polymer and their sequence determine the properties of that polymer</td>
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<td>LO 4.3</td>
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<tr>
<td>The student is able to use models to predict and justify that changes in the subcomponents of a biological polymer affect the functionality of the molecule.</td>
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<tr>
<td>LO 4.9</td>
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<tr>
<td>The student is able to predict the effects of a change in a component(s) of a biological system on the functionality of an organism(s).</td>
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<td>Outcomes &amp; Practices</td>
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<td><strong>LO 4.17</strong></td>
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<td>The student is able to analyze data to identify how molecular interactions affect</td>
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<td>structure and function</td>
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<td><strong>Science Practice 1:</strong></td>
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<td>The student can use representations and models to communicate scientific phenomena</td>
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<td>and solve scientific problems</td>
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<td><strong>Science Practice 2:</strong></td>
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<td>The student can use mathematics appropriately.</td>
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<td><strong>Science Practice 3:</strong></td>
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<td>The student can engage in scientific questioning to extend thinking or to guide</td>
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<td>investigations within the context of the AP course</td>
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<td><strong>Science Practice 4:</strong></td>
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<td>The student can plan and implement data collection strategies appropriate to a</td>
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<td>particular scientific question</td>
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<td><strong>Science Practice 5:</strong></td>
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<td>The student can perform data analysis and evaluation of evidence.</td>
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<td><strong>Science Practice 6:</strong></td>
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<td>The student can work with scientific explanations and theories</td>
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### Advanced International Certificate of Education (AICE) Biology Learning Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
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<td>C (b).</td>
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<td>Explain the mode of action of enzymes in terms of an active site, enzyme / substrate complex, lowering of activation energy and enzyme specificity.</td>
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<td>C (c).</td>
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<td>Follow the progress of an enzyme-catalysed reaction by measuring rates of formation of products or rates of disappearance of substrate</td>
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<td>Investigate and explain the effects of temperature, pH, enzyme concentration and substrate concentration on the rate of enzyme-catalysed reactions.</td>
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<td>C (e).</td>
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<td>Explain the effects of competitive and non-competitive inhibitors on the rate of enzyme activity</td>
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<td>I (b).</td>
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<td>Candidates will be able to describe the cause of HIV / AIDS</td>
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<td>I (c).</td>
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<td>Candidates will be able to explain how HIV / AIDS is transmitted</td>
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<td>I (d)</td>
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<td>Discuss the roles of social, economic, and biological factors in the prevention and control of HIV / AIDS.</td>
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<td>I (e).</td>
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<td>Discuss the global patterns of distribution of HIV / AIDS and assess the importance of these diseases worldwide</td>
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Background Information

General background information is given here. More information can be found within the individual lessons.

The human immunodeficiency virus (HIV) is the primary cause of acquired immune deficiency syndrome (AIDS). This pandemic has caused over 30 million deaths around the world, and is still one of the most serious global health problems that we face today. The HIV is transmitted from an infected individual most commonly through all types of unprotected sex or direct contact with other bodily fluids with a high viral load (such as blood). That being stated, it is important to note that contact with certain bodily fluids of an infected individual (such as tears or saliva) have not shown to communicate the disease.

Once an individual becomes infected with HIV, the immune system has a quick response to the growing infection, and symptoms will actually be very similar to the common flu soon after the initial exposure. Shortly thereafter, the individual will show signs of recovery, and flu like symptoms will subside. This is known as the “latent stage” of the infection, and the viral load is slowly increasing (assuming no treatments) in the body while CD 4+ T cells (immune cells) population will be declining. This stage can last for years before additional symptoms of the infection become clearly apparent. Eventually, with no treatment, the individual’s immune system is destroyed.

The origin of HIV stretches back into the 1930s from Central Africa, although it can now be found in all continents except Antarctica. There are now several strains of HIV, with the most viral genetic diversity being found in Cameroon and other Central African countries. Although HIV demonstrates amazing genetic diversity, HIV-1 (group M) is clearly the most widespread of all of the strains.

The most common treatment for an individual who is HIV positive is called “highly active antiretroviral therapy”, or HAART, and is a mixture of several different drugs that fight HIV in different ways. One of the most promising modes of treatment (see “A Mutant in Our Midst, Part 1” for full descriptions of the different treatments) is the protease inhibitors. HIV-1 protease is a molecule found in active HIV which allows the virus to successfully replicate. When this protease is inhibited, the virus is not able to replicate, which will lower the viral load in the individual. It is important to note that this protease can mutate, and commonly does. Many of the primary inhibitors of HIV-1 protease will not work in the mutant protein. Because of this, the area of HIV-1 protease inhibition is being widely researched. Part of this unit (lessons 4-6) mimics the process of drug discovery, development, optimization, and crystallization. These are the exact same steps that many researchers are currently pursuing in the hunt for the next HIV treatment drug.
LESSON ONE: “HIV? Are you positive?”

KEY QUESTION(S): How does an individual get HIV, and how does it turn into AIDS?

KEY SCIENCE CONCEPTS: HIV, AIDS, viral transmission, retrovirus, immune system.

OVERALL TIME ESTIMATE: This lesson will require around 55 minutes to complete.

LEARNING STYLES: Auditory

VOCABULARY:

AIDS - Acquired immune deficiency syndrome. Caused by HIV and is classified as “AIDS” when CD4+ T cell count is less than 200 cells/mm³.

Antibodies - Proteins that an individual’s immune system makes in order to fight an infectious agent.

Bushmeat - Product of the killing of wild animals (most commonly primates) for subsistence or commercial purposes.

CD 4+ T cells - One of the specific human immune system’s cells, or white blood cell, that HIV infects.

HIV- Human immunodeficiency virus - Retrovirus that is the cause of AIDS.

Lymph Nodes - Nodules in the lymphatic system where some white blood cells are produced, and lymph is filtered.

Lymphocytes - White blood cells.

Retrovirus - RNA virus that uses reverse transcriptase to integrate it genetic material into the host’s genetic material.

Reverse Transcriptase - The enzyme that retroviruses use to copy and insert their RNA into the host’s DNA.

Secondary Infections - Opportunistic infections that are the result of the HIV infection.

Seroconversion - The development of detectable amounts of HIV antibodies in the blood.

SIV - Simian immunodeficiency virus. Precursor to HIV and carried by apes and monkeys.

Virions - Fully-formed viruses that are traveling around the body.
LESSON SUMMARY: This lesson will focus on how HIV will affect the individual’s body. First, a modified roundtable activity will be used to discuss what is known about HIV. After this discussion, a case study will illustrate the modes of HIV transmission from human to human followed by a short lesson into how HIV can progress into AIDS.

STUDENT LEARNING OBJECTIVES:
The student will be able to...
1. Understand risk factors of HIV transmission along with being able to determine most likely routes of transmission.
2. Determine the stages of HIV/AIDS by understanding the relationship of CD4+ T cells to viral load.

STANDARDS
Next Generation Sunshine State Standards
SC.912.L.14.6
SC.912.L.16.7

Advanced Placement (AP) Biology Learning Outcomes:
LO 2.29
LO 3.29

Advanced International Certificate of Education (AICE) Biology Learning Outcomes:
I (b).
I (c).

MATERIALS:
- **ESSENTIAL** (Per group of 4 students):
  - 2 Markers
  - Poster Board or Butcher Block Paper
- **SUPPLEMENTAL**:
  - Additional markers for the groups

BACKGROUND INFORMATION:
Acquired immune deficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV). There are two types of HIV: HIV-1 which is more prevalent, and HIV-2 which is only found in West Africa. Both can cause AIDS, but unless otherwise noted, when “HIV” is referred to, HIV-1 is being specifically addressed. There are different subgroups of HIV-1, but this will be addressed in a different lesson (lesson 3). It is widely accepted that HIV came from a mutated version of the simian immunodeficiency virus (SIV), which is carried by apes and monkeys. African hunters would kill the monkeys for their bushmeat and would commonly come in contact with the infection.

The most current numbers from the World Health Organization (WHO) estimated that in 2010 there were 34 million individuals living with HIV (30.1 million adults, 3.4 million children under 15 years old), and 1.8 million individuals died from the infection. Although the treatments for the infection are becoming more effective and care for infected individuals is becoming better, HIV/AIDS is still #6 on the WHO’s “Top 10 Causes of Death” (around the world).
Although HIV will cause a certain set of symptoms, the timing and severity of these can vary depending on the individual. Interestingly, because of small genetic differences, there is a small subset of individuals who will not develop AIDS even if they have HIV. The main route for the spread of this virus is the transmission of bodily fluids through unprotected sex (of all types), sharing needles, blood transfusions, and from mother to child (this is most probable during labor or because of breastfeeding; however, with preventative measures, the risk can be minimized). Once infection occurs, HIV will begin to rapidly infect immune system cells (CD4 T cells) and the host will begin to produce large amounts of antibodies (seroconversion). The virus will replicate at the rate of $1 \times 10^{10}$ virions per day. These viruses will infect white blood cells and will lead to the destruction of 1-2 x $10^9$ of them on a daily basis. It is thought that this continual replacement of roughly 1% of the white blood cells in a human body on a daily basis leads to the eventual demise of the immune system and the progression to AIDS (for the progression in the human body, see below in the “specific topics that need to be taught”). It is not uncommon for a decade to pass before the HIV infection turns into AIDS.

ADVANCE PREPARATION:

1. Read through the background information, case study, and progression of HIV section of this lesson plan. Gather required material (noted above).

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. (15-20 minutes) Pre-Lesson Activity:
   a. Outline of activity:
      1. Instruct students to get out a piece of paper.
      2. Instruct students to take 5-10 minutes to write down what they know about HIV/AIDS.
      3. Divide students into groups of four. Give each group a poster board and markers.
      4. Instruct students to divide poster into 3 vertical sections labeled “Positively True”, “Positively Untrue”, and “Not Sure”.
      5. Have students transfer what they wrote onto the poster, but the group must come to a consensus on which column it should go into.
   b. Detailed description of activity:
      Instruct students to get out a piece of paper (it may be scrap paper). Introduce the idea that there is a lot of information about HIV/AIDS in the public, some of which is correct, and some of which is incorrect. Instruct students to take 5-10 minutes to write down what they know about HIV/AIDS (this may be done in a list format). At the end of this time, put students into groups of four. Have students divide a poster board into 3 vertical sections. Section 1 should be titled “Positively True”, section 2 should be titled “Positively Untrue”, and section 3 should be titled “Not Sure”. Instruct students to discuss their current HIV/AIDS knowledge with each other, and to transfer what they wrote on their individual lists to the poster board. It is essential that the group come to consensus before anything is written on the board. This should promote discussion about the topics- especially the topics that are not as well understood. Although the teacher can discuss what was written on the posters, it is assumed that some of the information on the posters will be incorrect. Any incorrect information should be noted as incorrect during the following activity.

2. (30 minutes) Case Study:
   a. Outline of activity:
      1. Keep student in same groups.
2. Tell students that they will be reading a case study about a sensitive topic.
3. Allow students adequate time to read case study.

Be sure to begin the case study by making clear to your students that as they read case studies about patients, there should be no judgment about how this individual’s behavior led to his contracting HIV. Patients always need to be treated with respect.

3. (10 minutes) Specific concepts about HIV that should now be covered/taught through a short lecture and PowerPoint:

ASSESSMENT SUGGESTIONS:

- Students should be able to make corrections on their posters. Depending on how many errors that were actually made, new poster boards or paper might have to be distributed. All of the questions associated with the lesson should be turned in.

EXTENSIONS:

- Although this case study is fictional, there are many case studies with questions that can be found at the National Center for Case Study Teaching in Science (http://sciencecases.lib.buffalo.edu/cs/collection/). Additionally, students could complete posters on the “Stage 4 Clinical Manifestations” of HIV (see above for list) that describe and explain the manifestation.

RESOURCES/REFERENCES:

Joe was your typical 30 year old medical student, although his last 4 years were anything but typical. Being recently diagnosed with Human Immunodeficiency Virus (HIV), Joe reflected on his activities during his time in medical school. Because he was a medical student who was commonly in the emergency room, he has had experiences that the average person will never have in a lifetime. The following is his story:

“Although it was confirmed that I have HIV, I am not completely sure on how I got it. Exposure could have come from all different things I was exposed to. I will begin my story four years ago, when I started rotations in the emergency room. I lived the typical life of a medical student—little sleep, hard work, stress, and little time for fun. I had a girlfriend for a short period of time close to the beginning of these four years, and we did engage in unprotected sex once or twice (I know, as a medical student I should have known better…). I did not think too much about it. I had known her for a long time, and to my knowledge, she did not have HIV (and to my knowledge, still does not have HIV).

“It was a fairly easy breakup, but it was on the drive home from this breakup that my life changed. I was driving fast, but I did not see the red light. I went right through it and blindsided a SUV at about 45mph. I was immediately knocked unconscious, and I don’t remember anything until I woke up in the hospital. My arm and hand (or, what I thought was my hand) was wrapped in a huge bandage and I had a cast on my leg. When the doctors came in, I was told that I had lost my hand in the accident, lost a great deal of blood and had to have a blood transfusion, broke my leg, received a concussion, and received other bumps and bruises. This was, of course, life changing news. As a medical student I knew that there was such a thing as a hand transplant (look it up—it is real!) and I might have a chance to receive one. If not, I knew I had no chance of actually becoming a surgeon one day. After a couple months on a waiting list, I received word that a donor hand was available and I needed to come in for the procedure. That was great news, and the procedure went as planned. I was not prepared for the recovery—it was long, and painful. In fact, it was so painful that my pain killing medication became a drug that I needed all the time. It began to lose its potency, and I moved onto stronger painkillers. These painkillers were intravenous (administered into a vein) and were not prescribed. I quickly became hooked, and was consumed with trying to
buy and find these painkillers. Even at the time, I knew that intravenous drug use was highly risky, but I just didn’t care. The battle with painkillers lasted nearly 5 months, but finally with the help of friends and family, I got off of them. That was the right move if I ever was going to finish medical school.

“Speaking of friends… I did meet a new girl during this time. Although it never became too serious, we shared some memories. That all came crashing down when I made my “move”, and gave her a kiss on the lips. She pushed me, and walked away. I felt pretty dumb at this point, but out of frustration and embarrassment, I let her go. When I called her the next day she informed me that two years ago she was diagnosed with HIV and she did not want a relationship because of it. I guess I respected that, but at the time, I was just frustrated with the news. To my knowledge, this was the first contact I ever had with someone who had HIV.

“So, I took a vacation. It was summer, and I love to camp and hike. I decided to go out on my own for a week- away from everyone else. Although the scenery was amazing, the mosquitoes were in full swing. I cannot count the number of mosquito bites I received, but it had to be over 100 within a week’s time. I knew mosquitoes could carry some parasites in their saliva, but I thought that actually obtaining one of these infections would be unlikely. I wonder if mosquitoes can carry HIV?

“Either way, I was finally heading back to medical school after my long hiatus to get well after the accident.. I was excited to be back in an emergency room learning about what I loved. Although the first week back was fairly standard, the next was anything but normal. I remember that a mother and toddler came in, both were crying. Although I was not an attending doctor, I was still in the area watching and learning. The toddler had a large cut on his leg, and was bleeding profusely. Everyone involved was wearing appropriate protection (gloves, gowns, face masks, etc...), but the toddler was squirming all over the place. Tears and snot seemed to be flying everywhere, it was clear the toddler was in a good deal of pain. As I went in to help control the child, my arm got close to the toddlers face, and apparently he felt it was appropriate to bite my arm (I am not sure if I blame him in his situation). Although the bite was painful, no skin was broken. After the bleeding on the child’s leg was controlled, we proceeded to clean up the blood spatters on the floor and linens. Before this clean up, I neglected to check my gloves. It was only after the cleanup that I noticed the hole in my glove. Although I cannot be sure, I am fairly certain the hole was there during part of the blood cleanup. I proceeded to thoroughly wash my hands and arms and made sure I had no open wounds on my hands. I did not find any cuts, and I went to discuss the event with the attending doctor. The doctor instructed me that both the toddler and the mother had HIV. Of course, the events of the day then replayed in my head- what about the bite? What about the glove? I did not discuss this with the doctor because all contact I might have had with HIV was because I was being neglectful. As a medical student, my goal was to always look like I had it under control- so I moved on. I visited the mother and child while they were still in the ER; the mother thanked me, shook my hand and said goodbye. Of course, in the retrospect of being diagnosed with HIV two years later, I have many more questions about this event.
“Oh, I almost forgot to mention ... I finally went through with it... I got a tattoo. I was not sure if I was ever going to get one, but I finally convinced myself that as long as it is not easily visible then no one in my profession would even know. I got a caduceus on my shoulder- that ever so famous medical symbol of the two snakes wrapped around a winged staff. The place I went to get it from seemed a little shady (it seemed clean, but just very odd people there), but it turned out well.

“The next couple months were fairly standard- hard work, long hours, stress, and another girlfriend. You might be thinking to yourself ‘wow, not another girl.’ Trust me, I was thinking the same. Either way, we met through mutual friends and started hanging out. One thing led to another and before I knew it I was in some sort of relationship. It became pretty serious and soon after she moved into my small apartment with me. We shared everything- a bathroom, my kitchenware, and even food. I had become wiser from previous relationships and tried to always have protection during sex (although I did forget once with her). One evening, I came home and she was gone. All of her stuff was moved out, and she left a note saying that she was ‘done and wanted to move on.’

“Although this was a heartbreaking surprise, I just tried to forget about it. On top of trying to deal with that, I came down with the flu about 1 week later. This just made it worse. I don’t ever remember having the flu this bad. I never received the swine flu vaccine, so I assumed that is what I had come down with. I stayed at home, and slowly recovered.

“It was six months later in mid-June when I noticed that there were posters around the hospital about National HIV Testing Day on the 27th of this month. I had never heard of this, but a group of us at the hospital thought it would be a good thing to do as an example to individuals in the hospital that were asking questions about the posters. I had some blood drawn from my arm, and went on with my day. It was to my surprise when I got the call that told me I was HIV positive. Of course my mind began to race as to how I actually got the infection and what it meant for my life. To this day I am not completely sure how I got HIV. Even though I was a ‘brilliant’ medical student, I made mistakes and had many chance happenings, but I will carry this virus with me for the rest of my life.”
STUDENT PAGES: QUESTIONS FOR HIV CASE STUDY

1. Joe could have come in contact with the HIV virus many different ways during these four years. Make a “ranked” list of the most likely ways Joe became infected (in order from most likely, to least likely).

2. Out of the most likely ways that Joe became infected, what did they all have in common?

3. If the last girl in the case study gave Joe HIV, do you believe that she should be legally held responsible?
4. What implications do you think this infection will have on Joe’s life?

5. Did Joe have any symptoms of HIV?

TEACHER PAGES: HIV CASE STUDY QUESTIONS - ANSWER KEY

1. Joe could have come in contact with the HIV virus many different ways during these four years. Make a “ranked” list of the most likely ways Joe became infected (in order from most likely, to least likely).

   Most likely ways he contracted HIV: Unprotected sex with the last girl in the story (this is the most likely way he became infected—it seemed Joe did not know much about this girl and he also developed flu-like symptoms soon after his sexual relationship with her). Intravenous drug use (he said that he was “consumed” with taking these drugs and perhaps some of the needles were used by other people). Unprotected sex with first girl in the story.
Less likely ways he contracted HIV - **Blood transfusion** (In America, blood is screened for infectious agents, but it was never mentioned what country he was living in. In the 1980s, thousands of Chinese individuals were infected with HIV from blood transfusions. See http://www.asiacatalyst.org/news/AIDS_blood_scandals_rpt_0907.pdf for more information on this topic). **Hand transplant** (if the organ being transplanted is infected with HIV, there is very good chance the person receiving the organ will get HIV. However, the screening process is comprehensive enough to make sure the donor would not have communicable diseases. See Jay A. Fishman, M.D., and Robert H. Rubin, M.D. *N Engl J Med* 1998; 338:1741-1751 for more information on this topic). **Tattoo** (if the tattoo shop is following standard sterilization protocol, then the chance of infection is practically zero. However, if the shop is not following protocols, then the chance of infection is much higher). **Living with the third girl** in the story (day to day, non-sexual contact with an infected individual cannot transmit HIV as long as no bodily fluids were transmitted. See http://www.cdc.gov/mmwr/preview/mmwrhtml/00030972.htm for a story of a child that was infected with HIV because of the living conditions, which is a very rare occurrence).

Ways he did NOT contract HIV (all information in this section is from http://www.cdc.gov/hiv/resources/qa/transmission.htm) – **Kiss on the lips** of the HIV positive girl (HIV contraction has never been tracked back to a kiss on the lips; however, it is possible to transmit HIV through a “French Kiss” if the individuals have any open sores in their mouths). **Mosquito bites** (HIV cannot be transmitted through a mosquito. All of the communicable diseases that mosquitoes carry are in their saliva, and the virus cannot, and will not, live there). **Bite from the toddler** (the chance of infection if the bite did not break the skin is zero. Although there have been some documented cases of HIV transmission through a bite, these bites caused severe tissue damage with a great deal of bleeding). **Hole in the glove** (because Joe did not have any wounds on his hands, the chance of transmission is zero. The virus does not live outside of the body and the blood he came in contact with had been on the floor/bedding). **Tears/snot from the toddler** (the individuals that were attending to the child were all wearing appropriate protection. It can be assumed that no bodily fluid from the child entered into Joe). Even if it came in contact with Joe, the likelihood of infection from these specific fluids would be extremely unusual.

2. Out of the most likely ways that Joe became infected, what did they all have in common?
   They were all based on choices Joe made and not based on chance occurrences.

3. If the last girl in the case study gave Joe HIV, do you believe that she should be legally held responsible?
   Although this will come down to a student’s individual opinion, there are documented cases of individuals not telling their partners that they were infected with HIV. Charges have varied depending on what country it occurred in. Charges include (but are not limited to)
manslaughter, assault, attempted murder, and even first-degree murder. For more information, see http://www.aidsmap.com/law.

4. What implications do you think this infection will have on Joe’s life?
   
   Joe could still finish medical school, if he chooses to. Depending on his treatment plan, he might still live a long and successful life. If he chooses to not follow a prescribed medical plan, then his life expectancy will be shortened. Ultimately, he will carry the virus for the rest of his life.

5. Did Joe have any symptoms of HIV?

   Commonly, the individual will be asymptomatic (not have any symptoms) for 5 or 10 years. It is common that after initial exposure (within a couple weeks) the individual will come down with flu-like symptoms as the HIV replicates in the blood. Flu-like symptoms soon disappear, and usually the individual will not suspect a HIV infection.
The progression of HIV in the human body begins with receiving the virus and then continues to acquired immune deficiency syndrome (AIDS). The steps of this progression should be summarized as below in the associated PowerPoint (all material is from http://www.avert.org/stages-hiv-aids.htm, http://www.niaid.nih.gov/topics/HIV/AIDS/Understanding/Biology/pages/clinicalcourse.aspx, http://www.who.int/hiv/pub/guidelines/clinicalstaging.pdf). It would be ideal to have students create an outline of the progression of HIV into AIDS and also create a graph showing the relationship of CD4 T cells to HIV viral load.

A. Exposure to virus (see case study above). This is most commonly from exchanges of bodily fluids.

B. Primary/Acute Infection. This is the stage Joe experienced when he had flu-like symptoms. The HIV is replicating quickly in the blood and infecting helper T cells (specifically CD4 T) and the body is responding with the production of antibodies (a blood protein produced to counteract HIV) and more lymphocytes. The CD4 T cell count is dropping from the individual’s baseline value (value before infection). Because HIV is a retrovirus, it will copy its genetic material and insert it into the host cells genetic material (using reverse transcriptase). Most individuals will not even go to the doctor with these symptoms, and even if they did go, the doctor will very rarely consider HIV as a diagnosis. A common HIV test will not detect an infection at this stage. The initial symptoms subside and viral load drops, and CD4 T returns to almost baseline value.

C. Clinically Asymptomatic/Latent Stage. The individual might go 10 years free from major symptoms. The viral load will be low in the blood, but the antibodies can be detected by an HIV test. The HIV will be active in the lymph nodes. CD4 T levels slowly decline.

D. Symptomatic HIV Infection. By this point, the immune system is severely damaged. The CD4 T count is decreasing because they body has slowed down producing new ones, and the lymph nodes are deteriorating (functionally). Because of the decrease in CD4 T cells, the body is more susceptible to other infections and cancers. Besides the treatments being given for HIV, specific treatments also need to be given for the other infections. Viral load is slowly increasing.

E. Progression from HIV to AIDS. With modern testing, it is easy to establish an exact CD4 T count. If the count is lower than 200 cells/mm³ (normal count is 500-1000 cells/mm³) then the individual is considered to have AIDS. Life expectancy is decreased but will depend on the decline of CD4 T and exposure to secondary infections (these secondary infections often prove to be terminal. Tuberculosis is one of the most common secondary infections). Viral load is increasing. In underdeveloped countries, these tests may not be available. In this situation, the World Health Organization has established a “Clinical Stage List.” If an individual has the following symptoms (it can be decided by the instructor if details should be added to the following material), then they are categorized as “Stage 4”, and have AIDS:

- HIV wasting syndrome
- Pneumocystis pneumonia
- Recurrent severe or radiological bacterial pneumonia
- Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month’s duration)
- Oesophageal candidiasis
- Extrapulmonary TB
- Kaposi’s sarcoma
- Central nervous system (CNS) toxoplasmosis
- HIV encephalopathy

Conditions where confirmatory diagnostic testing is necessary:
- Extrapulmonary cryptococcosis including meningitis
- Disseminated non-tuberculous mycobacteria infection
- Progressive multifocal leukoencephalopathy (PML)
- Candida of trachea, bronchi or lungs
- Cryptosporidiosis
- Isosporiasis
- Visceral herpes simplex infection
- Cytomegalovirus (CMV) infection (retinitis or of an organ other than liver, spleen or lymph nodes)
- Any disseminated mycosis (e.g. histoplasmosis, coccidiomycosis, penicilliosis)
- Recurrent non-typhoidal salmonella septicaemia
- Lymphoma (cerebral or B cell non-Hodgkin)
- Invasive cervical carcinoma
- Visceral leishmaniasis

Figure 1. Progression of HIV over time. (From S. Dewhurst, R. L.W. da Cruz and L. Whetter. Frontiers in Bioscience, 5, d30-49 (January 1, 2000).

- This chart should be shown to the class after students have created their graph to compare their work versus actuality. The aforementioned stages should be related to the graph
1. What are some characteristics of the pathogenic agent HIV and how does it impacts humans?

2. How does HIV move around the body? What cells do they infect?

3. Draw a simplified version of the chart showing the progression of HIV over time in relation to CD 4⁺ T cells and viral load.

4. What happens to the HIV’s genetic material? What enzyme is used to turn HIV’s RNA into DNA?

5. What makes HIV and AIDS different? What is the cause of each?

6. What are the major communication routes of HIV?
1. What are some characteristics of the pathogenic agent HIV and how does it impacts humans?
   *HIV is a retrovirus that infects human immune cells. The virus slowly destroys the host immune system, making the individual more likely to be infected by a secondary pathogen.*

2. How does HIV move around the body? What cells do they infect?
   *It will move in the circulatory and lymphatic systems. Commonly, the virus infects CD4⁺T cells.*

3. Draw a simplified version of the chart showing the progression of HIV over time in relation to CD4⁺T cells and viral load.
   *See chart above.*

4. What happens to the HIV’s genetic material? What enzyme is used to turn HIV’s RNA into DNA?
   *HIV’s genetic material is incorporated into the host’s DNA by the actions of reverse transcriptase (and integrase).*

5. What makes HIV and AIDS different? What is the cause of each?
   *HIV is just the virus. AIDS is the clinical diagnosis of an individual who has a CD4⁺T cell count lower than 200 cells/mm³.*

6. What are the major communication routes of HIV?
   *Unprotected sex
   Intravenous drug use*
LESSON TWO – “SO MANY STRAINS, SO LITTLE TIME”

KEY QUESTION(S): What impact does HIV have on the world’s population? Where did HIV come from and how many different types are there?

KEY SCIENCE CONCEPTS: HIV, AIDS, phylogenetic tree, South Africa, World HIV maps.

OVERALL TIME ESTIMATE: This lesson will require about 100 minutes to complete. Can easily be split into multiple days (a good breaking point is after “Activity 1, World Mapping”).

LEARNING STYLES: Auditory and the use of technology

VOCABULARY:

AIDS denialists - Group of individuals who deny that HIV is the cause of AIDS.

Antiretroviral drugs - Drugs intended to fight retroviruses, such as HIV.

Circulating recombinant forms (CRF) - Forms of HIV that are a combination of the general subtypes.

Divergent evolution – The accumulation of differences in a species based on the physical separation of the species.

Durban Declaration - Article published in Nature that was intended to encourage Thabo Mbeki to condone antiretroviral treatments for those with HIV/AIDS.

Kgalema Motlanthe - President of South Africa from 2008 – 2009; promoted the treatment of HIV with antiviral drugs.

Pandemic - Worldwide epidemic.

Thabo Mbeki - President of South Africa from 1999 –2008; had controversial view of fighting HIV/AIDS with antiretroviral medicine.

LESSON SUMMARY: This lesson will focus on the geographic origin of HIV and how it mutated and moved to different parts of the world. Students will read part of a scientific paper to create a phylogenetic tree and use the Internet to construct world maps showing the frequency of different HIV strains around the world. Additionally, students will learn about an exceptionally strange situation in which the South African government denied that HIV was the cause of AIDS and how the world responded.

STUDENT LEARNING OBJECTIVES:

Student will be able to...

1. Describe the spread of HIV around the world and trace the virus back to its hypothetical geographic origin.
2. Explain the origins of HIV and depict the phylogenetic connections of different HIV strains around the world.
3. Understand the history of HIV in South Africa and be able to support their opinions of what should have been done differently to prevent the HIV/AIDS pandemic.
STANDARDS

Florida Next Generation Sunshine State Standards (NGSSS):
SC.912.L.14.6
SC.912.L.16.10

Advanced Placement (AP) Biology Learning Outcomes:
LO 1.17
Science Practice 1:
Science Practice 5:
Science Practice 6:

Advanced International Certificate of Education (AICE) Biology Learning Outcomes:
I (d)
I (e)

MATERIALS:

- ESSENTIAL:
  - Computer paper or graph paper for each student
  - A blank World Map print-out (link can be found below)
  - Access to a computer with Internet access.

BACKGROUND INFORMATION: HIV and AIDS can now be found around the world and in every continent (besides Antarctica). The prevalence of different types of HIV varies depending on the country. Overall, HIV can be split into the following groups (does not include "circulating recombinant forms" or CRFs of HIV. Example full phylogenetic tree can be seen in Figure 1)

ADVANCE PREPARATION:

1. Gather required materials (see above).
2. Make sure computers have Internet access.
3. Block out “Figure 2” from the paper found at http://www.stat.wisc.edu/~larget/Genetics629/hiv.pdf
4. Make a class-set of this paper (copy paper with blocked out “Figure 2”).
5. Print world map (available from http://www.freeworldmaps.net/printable/printable-world-map.gif, but any equivalent can be used).

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. (40 minutes) Activity 1. World Mapping of HIV/AIDS.
Each student should receive a world map and should have access to a computer with Internet access. Students need to log onto [http://www.hiv.lanl.gov/components/sequence/HIV/geo/geo.comp](http://www.hiv.lanl.gov/components/sequence/HIV/geo/geo.comp). This will bring up a world map showing the prevalence of HIV/SIV. Make sure to note that this map is only showing the frequency of HIV/SIV in specific areas, and not the population, around the world. The pie chart under the map (and to the right) shows the different types of HIV. There are multiple options that can be chosen under the map (to the left). Make note that the “organism” can be changed from HIV-1 to HIV-2 or SIV.

2. (40 minutes) Activity 2. Phylogenetic tree creating based on Scientific Literature.

In this assignment, students will read a portion of the article entitled: “THE CAUSES AND CONSEQUENCES OF HIV EVOLUTION”, by Andrew Rambaut, David Posada, Keith A. Crandall and Edward C. Holmes, Nat. Rev. Genet. 2004 Jan; 5(1):52-61 (can be found at [http://www.stat.wisc.edu/~larget/Genetics629/hiv.pdf](http://www.stat.wisc.edu/~larget/Genetics629/hiv.pdf)). The instructor must block out “Figure 2: Evolutionary history of the primate lentiviruses”, because students will create an abbreviated version of it based on the text. In no way is it expected that students should be able to create the entire phylogenetic tree or include the SIV strains.

Students should be able to deduce that HIV-1 and HIV-2 came from different ancestors, there are three groups of HIV-1 (M, which is the “major” strain of HIV; N, which is Non-M; and O, which is an outlier), there are nine subgroups of HIV-1 and seven subgroups of HIV-2. All of the information to do this can be deduced by students reading the introduction, “The Origins of HIV”, (specifically “Molecular phylogenies of HIV and SIV”), and “Box 1: HIV/AIDS: History and diversity). After students have created their phylogenetic trees individually, have them compare their work to their neighbor’s work. Allow for modification of their phylogenetic trees.

Once they are content with their work, show students “Figure 2: Evolutionary history of the primate lentiviruses” as it appears in the publication. Students will be confused with the addition of SIV strains, but point out HIV-1 and HIV-2 (and how they are not directly related by a common ancestor) on the phylogenetic tree along with their subtypes. Students do not need to read the entire article, but answering the following questions from their respective locations in this article does allow for a better understanding of the history of HIV.

Steps of this activity:

Students should:

1. Get a copy of “THE CAUSES AND CONSEQUENCES OF HIV EVOLUTION” with figure 2 blocked out.
2. Attempt to create a phylogenetic tree of HIV.
3. Compare their hypothesis to their neighbors, and any changes are allowed to be made
4. Check their work versus what is actually in the paper
5. Read remaining parts of article and answer the associated questions.

3. (30 minutes) Activity 3. Case Study: South Africa

a. Can be completed at home but plan for a 30 minute post discussion in class.

Sometimes truth is stranger than fiction. This website tells the true story of the HIV/AIDS epidemic in South Africa during the 1990s and how the world responded to the crisis. Have students go to [http://www.journaids.org/index.php/essential_information/the_politics_of_hivaids_in_south_africa/](http://www.journaids.org/index.php/essential_information/the_politics_of_hivaids_in_south_africa/) and
click on “The Politics of HIV in SA.” A drop-down menu will show up. Students should write a summary of the history of HIV/AIDS in South Africa which includes some of the highlights from this website (not all the information should be required). In addition to this, have students write a one paragraph response to learning the history of HIV in South Africa, what you think the impact was on South Africa, and what should be done next. Emphasize the fact that students can agree or disagree with how South Africa dealt with the epidemic. Once students complete this and return to class, encourage discussion of what they wrote. It is vital to make sure that the discussion is culturally sensitive (i.e., the South African government did not really have a reason to trust pharmaceutical companies from the Western world). As interesting notes, point out that current studies have shown that over 300,000 individuals in South Africa passed away directly because of Mbeki’s policies and lack of acceptance of antiretroviral drugs and possibly read part (or all) of the Durban Declaration to the class (links were found above in “Background”).

Steps of this activity.

Students should:

2. Click on “The Politics of HIV in SA”
3. Write a summary of the history of HIV/AIDS in South Africa
4. Write a one paragraph response to learning the history of HIV in South Africa, what you think the impact was on South Africa, and what should be done next.

The information on the website focuses on the history of HIV in South Africa, but it is important to note what is currently happening in South Africa to summarize the lesson. Although the rate of infection is higher there than anywhere else in the world (as noted in a previous section of this curriculum), the rate of infection is actually dropping in some populations (see [http://data.unaids.org/pub/Report/2008/20080904_southafrica_anc_2008_en.pdf](http://data.unaids.org/pub/Report/2008/20080904_southafrica_anc_2008_en.pdf) or for more information). This is encouraging news, but still many lives need to be saved.

**ASSESSMENT SUGGESTIONS:**

- There should be multiple products for this lesson (Map product and associated questions, phylogenetic tree and associated questions, South African timeline, response to South African HIV history).

**Florida Next Generation Sunshine State Standards (NGSSS):**

1. Students explained the significance of pathogenic agents (HIV) to the health of a population (South Africa) in their written response.
2. Students evaluated the impact of biotechnology (drug development and deployment in South Africa) on the individual and society when discussing the topic in class and when writing their responses.

**Advanced Placement (AP) Biology Learning Outcomes:**

1. Students were able to create a phylogenetic tree and answer questions about evolutionary history and shared characteristics.
2. Students created a phylogenetic tree to communicate scientific data and solve problems.
3. Students performed data analysis during the creation of their phylogenetic tree, and when interpreting pie charts on the world map assignment.
4. Students worked with scientific explanations and theories by reading the required article and answering the corresponding questions.

Advanced International Certificate of Education (AICE) Biology Learning Outcomes:

1. Students discussed the role of poverty and how it related to HIV and AIDS.
2. Students created a world map showing the distribution of HIV/AIDS around the world

EXTENSIONS:

• Assigned different groups of students a specific subtype of HIV to research. Topics could include prevalence, locations, and history.

RESOURCES/REFERENCES:

• [http://www.avert.org/hiv-types.htm](http://www.avert.org/hiv-types.htm)
• [http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html](http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html)
Step 1. Log onto http://www.hiv.lanl.gov/components/sequence/HIV/geo/geo.comp. This will bring up a world map showing the prevalence of HIV/SIV. Make sure to note that this map is only showing the frequency of HIV/SIV in specific areas, and not the population, around the world. The pie chart under the map (and to the right) shows the different types of HIV. There are multiple options that can be chosen under the map (to the left). Make note that the “organism” can be changed from HIV-1 to HIV-2 or SIV.

Step 2. List (directly on the world map) the 3 most prevalent types of HIV-1 that can be found in each of the following continents: Asia (can be subdivided into smaller regions, Africa, North America, South America, Europe, and Australia. Do NOT include the recombinant forms of the virus. Click show “non-recombinant.”

Questions:
1. Compare the diversity of HIV-1 in Africa versus North America. Hypothesize an explanation of why there is so much genetic diversity of HIV-1 in Africa?

5. What countries in Africa have the most genetic diversity of HIV-1? HIV-2? (when zoomed in on Africa, click on “select organism” to change the virus and then click show “non-recombinant”)

6. What countries in Africa have the most genetic diversity of SIV (Simian Immunodeficiency Virus, or SIV, is the monkey equivalent of HIV in humans). When zoomed in on Africa, click on “select organism” to change the virus to “SIV” and then click show “all”)

7. Based on the information collected in the previous questions, what area in Africa did HIV most like originate from? Why do you think this?
8. In what countries in Africa could I find the highest prevalence of HIV-1 type “O”? Why do you think it is only found in this area of the world?

9. Is there more genetic diversity in HIV-1 or HIV-2? Suggest a reason why this is.

   **Step 3.** Go to [http://together.until.org/hivaidsepidemicstats_world.php](http://together.until.org/hivaidsepidemicstats_world.php) and add number of individuals who have the infection (by continent, or area) to your map.


10. Where in the world is HIV most prevalent (make sure “Prevalence of HIV, total (% of population ages 15-49” is selected)?

   **Step 5.** Go to [http://aidsvu.org/map](http://aidsvu.org/map).

11. In what areas of the USA is HIV most prevalent?

12. Change the map settings so it will only show ages 13-24. What noticeable changes are there on the map?

13. Change the map so you are also comparing it to poverty (change setting in “Social Determinants of Health”). Do you think there is a connection between poverty and HIV? Why?
1. Compare the diversity of HIV-1 in Africa versus North America. Hypothesize an explanation of why there is so much genetic diversity of HIV-1 in Africa?

_HIV-1 is much more diverse in Africa versus North America. Their hypotheses will vary, but the most likely response will be that HIV-1 possibly originated in Africa and has had longer to mutate. Other possible answers might include isolated populations (founder effect) have allowed the viruses to independently mutate._

2. What countries in Africa have the most genetic diversity of HIV-1? HIV-2? (when zoomed in on Africa, click on “select organism” to change the virus and then click show “non-recombinant”)

_HIV-1. Cameroon, Equatorial Guinea, and Gabon
HIV-2. Equatorial Guinea, Ghana, Cote D’Ivoire, Liberia, Sierra Leone

3. What countries in Africa have the most genetic diversity of SIV (Simian Immunodeficiency Virus, or SIV, is the monkey equivalent of HIV in humans). When zoomed in on Africa, click on “select organism” to change the virus to “SIV” and then click show “all”)

_Cameroon, Dem Rep of Congo, Gabon

4. Based on the information collected in the previous questions, what area in Africa did HIV most likely originate from? Why do you think this?

_Central Africa, around Cameroon. The most diversity of viruses can be found there._

5. In what countries in Africa could I find the highest prevalence of HIV-1 type “O”? Why do you think it is only found in this area of the world?

_Cameroon, Equatorial Guinea, and Gabon. It is most likely a new type of HIV._

6. Is there more genetic diversity in HIV-1 or HIV-2? Suggest a reason why this is.

_HIV-1. Students may suggest that it has been around for a longer period of time, although the exact dates are not known. Some sources say that HIV-2 is more difficult to transmit and symptoms take longer to develop._

Step 3. Go to http://together.until.org/hivaidsepidemicstats_world.php and add number of individuals who have the infection (by continent, or area) to your map.

7. Where in the world is HIV most prevalent (make sure “Prevalence of HIV, total (% of population ages 15-49” is selected)?
South Africa

Step 5. Go to http://aidsvu.org/map.

8. In what areas of the USA is HIV most prevalent?
In highly populated areas, by the coasts.

9. Change the map settings so it will only show ages 13-24. What noticeable changes are there on the map?
It seems that the Southeast United States (especially South Florida) has high HIV infection rates for ages 13-24.

10. Change the map so you are also comparing it to poverty (change setting in “Social Determinants of Health”). Do you think there is a connection between poverty and HIV? Why?
There is a direct relationship between poverty and HIV infections.
**Step 1.** Create a phylogenetic tree on computer/graph paper based on the information found in the introduction, “The Origins of HIV”, (specifically “Molecular phylogenies of HIV and SIV”), and “Box 1: HIV/AIDS: History and diversity.

*General article questions:*

1. What is the best type of evidence to use to ensure that this phylogenetic tree is accurate?

2. According to “Figure 2”, what are the newest types of HIV?

   *“Dating the evolution of primate lentiviruses”*

3. What is the definition of “molecule clock”?

4. In what year is it thought that HIV-1 (group M) and HIV-2 originated?

5. What should we look at to observe the timescale of HIV?

   *“Box 2: Analyzing rates of nucleotide substitution in HIV”*

6. How confident are the authors in the accuracy of the data in “Box 2”?

   *“Emergence of the human diseases”*

7. Why do the authors think that SIV jumped to humans many times before the world’s current epidemic?

8. How genetically different are the HIV-1 subtypes.

9. What HIV-1 subtype is most common in North America and when is it thought to have originated?
“Evolutionary processes” (just the first paragraph of "Within-host evolution")

10. What is the one characteristic that makes HIV so unique and what two factors is it caused by?
General article questions:

1. What is the best type of evidence to use to ensure that this phylogenetic tree is accurate?
   Molecular and genetic.

2. According to “Figure 2”, what are the newest types of HIV?
   All of the current types that we currently find in the human population.

“Dating the evolution of primate lentiviruses”

3. What is the definition of “molecule clock”?
   “The principle that any gene or protein has a near-constant rate of evolution in all branches of a clade, which means that the amount of sequence divergence between two sequences will be proportional to the amount of time elapsed since their shared ancestor existed.”

4. In what year is it thought that HIV-1 (group M) and HIV-2 originated?
   1930s

5. What should we look at to observe the timescale of HIV?
   Archival HIV samples

“Box 2: Analyzing rates of nucleotide substitution in HIV”

6. How confident are the authors in the accuracy of the data in “Box 2”?
   Although the authors bring up that some doubt the accuracy of the data, they recognize it is the “best estimate so far.”

“Emergence of the human diseases”

7. Why do the authors think that SIV jumped to humans many times before the world’s current epidemic?
   Many humans came in contact with SIV and it is thought that the infection “burnt out” because of the remote locations of the infected individuals.

8. How genetically different are the HIV-1 subtypes?
   10-30%

9. What HIV-1 subtype is most common in North America and when is it thought to have originated?
   Subtype B, 1960s or 1970s
“Evolutionary processes” (just the first paragraph of "Within-host evolution")

10. What is the one characteristic that makes HIV so unique and what two factors is it caused by?

   *It is one of the fastest evolving organisms because 1. High mutation rate and 2. Fast reproduction.*
Go to: http://www.journaids.org/index.php/essential_information/the_politics_of_hiv_aids_in_south_africa/ and click on “The Politics of HIV in SA.” A drop-down menu will show up. Write a summary of the history of HIV/AIDS in South Africa which includes some of the highlights from this website (not all the information should be included). In addition to this, write a one paragraph response to learning the history of HIV in South Africa, what you think the impact was on South Africa, and what should be done next.
FIGURE 1 – PHYLOGENETIC TREE

- HIV-1
  - Group M (Major)
    - Subtype (clade) A. West and Central Africa, Russia.
    - Subtype (clade) B. Most common subtype in the Americas, Europe, Australia, and Japan.
    - Subtype (clade) C. East and South Africa, India. Responsible for about half of all worldwide HIV infections.
    - Subtype (clade) D. East and Central Africa.
    - Subtype (clade) F. Central Africa, South America, Eastern Europe.
    - Subtype (clade) G. Central Europe, East and West Africa.
    - Subtype (clade) H. Central Africa.
    - Subtype (clade) J. Central America.
    - Subtype (clade) K. Central Africa.
  - Group N (New, non-major, found in Cameroon and is rare)
  - Group O (Outlier in West-Central Africa)
  - Group P (Outlier, only one reported infection in Cameroon)

- HIV-2
  - Group A- West Africa
  - Group B- West Africa
  - Groups C-H- All dead-end infections found in just one person.
It is important to specifically take notice of HIV-1 M subtypes “B” and “C”. Although HIV-1 B is prevalent here in North America, it is not the most prevalent worldwide (HIV-1 C is the most prevalent5). It is clear that Africa has the most genetic diversity of HIV-1 in the world, possibly due to the fact it originated there and continues to mutate.9 Most of the genetic diversity is located in Central Africa, specifically in Cameroon (the suspected location of the virus origin4), but the highest rate of infection (worldwide) is South Africa.5 HIV-2 is very closely related to SIV infections, but is not as widespread as HIV-1.5

It is thought that the common ancestor to the subtypes of HIV-1 M existed around 1930,7 but the oldest specimen is from 1959 (from the Republic of Congo).3 HIV-1 M, subtype B (most common in North America) is thought to have originated around 1960.8 There are many different reasons why there is so much diversity in HIV now, including divergent evolution, mutation rate, and speed of viral replication.3 New CRFs are showing up on a regular basis.9 HIV-1 infections in North America are most commonly found in highly populated areas and the poverty rate has a direct relationship to HIV frequency.10-11
In this curriculum, South Africa is specifically looked at as an example of how certain parts of the world have a difficult time accepting Western medicine, and what effects that can have on a population. It is important to familiarize yourself with the story using the same resource that the students will be using: (http://www.journaids.org/index.php/essential_information/the_politics_of_hivaids_in_south_africa/), but a summary can be found here as well.

HIV infections were becoming more and more prevalent in South Africa (and around the world) in the 1990s and the South African government (specifically president Thabo Mbeki) became skeptical of endorsing antiretroviral drugs. His skepticism grew and he became associated with AIDS denialists (President Mbeki placed several denialists on his advisory boards). The government pushed “natural treatments” for the treatment of those with AIDS. Scientists who had a background in HIV and AIDS knew that the further delay of these drugs would cause the pandemic to continue and grow. Because of this, the “Durban Declaration” was written and published in the scientific journal Nature. This declaration stated that HIV was the cause of AIDS and encouraged the pursuit of drug treatments for those who were infected (the actual document can be found at http://www.nature.com/nature/journal/v406/n6791/full/406015a0.html and http://www.survivreausida.net/article4150.html). It was signed by 5,000 doctors and researchers. Although it did not seem that this declaration had any effect on President Mbeki, it is none the less an important document showing worldwide unity in the support of HIV and AIDS treatment. In 2008, president Mbeki resigned his position to Kgalema Motlanthe. President Motlanthe supported the use of medicine to treat HIV/AIDS, but the roll-out of drugs has been slower than hoped. Currently, the infection rate in certain groups of South Africans is declining for the first time in recent history.
LESSON THREE: “A MUTANT IN OUR MIDST, PART 1”

KEY QUESTION(S): How are medications developed? How is HIV/AIDS treated?

KEY SCIENCE CONCEPTS: HIV, AIDS, drug development, drug groups.

OVERALL TIME ESTIMATE: 60 minutes

LEARNING STYLES: Use of technology, kinesthetic

VOCABULARY:
HAART - Highly Active AntiRetroviral Therapy. The most commonly used drug combinations to fight HIV/AIDS.

High-throughput technology - Technology that allows high-speed, automated testing of samples.

in vitro - In test tubes and petri dishes.

in vivo - In living organisms.

Lead compounds - The most likely drug candidates that will be investigated.

Molecular entities - Potential drugs

Nucleotides - Fundamental building blocks of DNA (adenine, thymine, guanine, cytosine).

Polypeptide - A polymer (chain) of amino acids

Reverse transcriptase - Enzyme that HIV uses to turn its RNA into DNA that is then inserted into the hosts DNA.

Target molecule - The molecule that is not working properly, or needs to be inhibited. This molecule is the target of the drug in development.

LESSON SUMMARY: In “A Mutant in Our Midst, Part 1”, students will learn general information about how medicinal drugs are developed and researched. Students will complete an investigation into what types (groups) of medicines are available to treat HIV/AIDS infections and how they generally work.

STUDENT LEARNING OBJECTIVES:
Student will be able to....

1. Create a timeline of the drug discover/development process.
2. Create an outline that demonstrates how the different classes of HIV drugs work.

Florida Next Generation Sunshine State Standards (NGSSS) for parts “1 and 2”:
SC.912.L.16.10
SC.912.L.18.4

Advanced Placement (AP) Biology Learning Outcomes for parts “1 and 2”:
LO 4.1
LO 4.2
MATERIALS:

- **ESSENTIAL:**
  - Computer with projector.
  - Drug Discovery cards, 1 set per student group
  - Student Handout: Drug Discovery and Development, 1 per student

BACKGROUND INFORMATION: The drug discovery/development process is long, expensive, and requires the work of a huge number of people. It is important that students understand the basic steps of this process. Outlined below is a breakdown of this process.

1. **Pre-Drug Discovery** - The disease/disorder is researched. The cause(s) and target molecule are identified.
2. **Drug Discovery** - Up to 10,000 new molecular entities are identified and tested with high-throughput technology. Medicinal chemists optimize the structure of the molecules. Products are called lead compounds, and are the most likely molecules (from the new molecular entities) to be usable drugs.
3. **Preclinical Drug Development** - Lead compounds are tested for safety, toxicity, dosage, and stability for humans (testing is not done in the human). Tests are completed both in vivo and in vitro. Usually only 250 molecules are found to still be useful.
4. **Submitted to Food and Drug Administration (FDA) as an Investigational New Drug (IND)** – FDA will determine if it is safe to be tested in humans.
5. **Clinical Trials**
   a. **Phase 1** - A few healthy patients (could be less than 30) are given the drug to determine safety and dosage.
   b. **Phase 2** - A few sick patients (around 100) are given the drug to determine safety, dosage, and if it works to combat the targeted disease or condition.
   c. **Phase 3** - A large group (thousands) of sick patients are given the drug to determine safety, dosage, and if it works. Usually only 1 drug out of the potential 10,000 new molecular entities remains at this point of testing.
6. **Submission for Market Approval** - The drug company submits all records of the drug to the FDA for market approval. FDA makes final decision if the drug can be manufactured, cannot be manufactured, or if further research needs to be done.
7. **Manufacturing** - The pharmaceutical company begins the process of manufacturing large quantities of the drug.

It is evident that much time and money must be used to complete the aforementioned steps. It is not uncommon for the process to take anywhere from 10-15 years, but it could even take 30 years before a marketable product is approved.\(^1\) It has been reported that the average drug costs anywhere from $55 million to $2 billion to develop and test\(^2,3\), but there is a huge amount of debate over the actual cost of drug discovery and development in the Western world.\(^4,5\) As the debate rages forward, drug developers in India and China are making great strides forward by offering more affordable drugs (usually “generic” drugs, but still effective) to some of the most underprivileged around the world.\(^6\)
There are several classes of drugs that are used to treat HIV/AIDS. In most cases, these drugs are used in a very specific combination. The use of combined drugs (or “cocktails”) is commonly called “highly active antiretroviral therapy”, or HAART. The use of HAART can help preserve the individual’s health by keeping the viral count below detectable levels. As with all HIV/AIDS treatment strategies, adherence to the prescribed program is vital to the longevity of its effectiveness. If an individual stops taking the medications, or even takes them improperly, it is highly likely that drug resistant strains will develop in that individual. Although there are subtypes of many of these drug classes, general explanations of each can be found below:

- **Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTI):** NRTIs interfere with the action of an HIV protein called reverse transcriptase. The NRTI is a molecule similar to the nucleotides normally found in DNA, but when they are added to the DNA strand being synthesized (from the viral RNA), the DNA replication process terminates.
- **Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI):** NNRTIs also stop HIV from replicating within cells by directly inhibiting the reverse transcriptase protein.
- **Protease Inhibitors (PI):** PIs inhibit protease, which is protein involved in the normal HIV replication process. Protease cleaves (cuts) a viral polypeptide to create some of the necessary proteins in HIV.
- **Fusion or Entry Inhibitors:** Fusion or entry inhibitors prevent HIV from binding to or entering human immune cells.
- **Integrase Inhibitors:** Integrase inhibitors interfere with the integrase enzyme, which HIV needs to insert its genetic material into the human’s DNA.

**ADVANCE PREPARATION:**

- Cut out the “Drug Discovery” cards
- Make copies of the Student Handout, Drug Discovery and Development

**PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:**

(30 minutes) Drug Discovery Process Timeline and Video:

1. Cut out the Drug Discovery cards (print on cardstock and/or laminate card for continued use). Have students put the name with a specific description, and put them in the correct order.

2. Have students answer the “student questions” before you check what order they have them in, or watching the video.

3. Go to [http://www.innovation.org/index.cfm/insidedrugdiscovery](http://www.innovation.org/index.cfm/insidedrugdiscovery), scroll down to “condensed version of the video series”. A video will pop-up. Have students watch the video and make any corrections to their work (both the order/names of the cards and “student questions.”

4. After the class watches the video, lead a short class discussion that compares what they wrote to how the process actually functions (possibly take a class poll on who guessed “too low” or “too high”.

(30 minutes) Groups of Medications to Treat HIV:

1. Hand out the student worksheet entitled “HIV Medication Types”.

2. Have students work in groups to try and determine the function of the drug group just by the name. Discuss student’s answers and add correct answers to board.
3. Write all the drug group names on the board.

4. After 10 minutes, ask the class what they came up with. If there is a correct answer, add it to the board.

5. If the class does not know the answer to one of them, try to break apart the word (of the drug group name) into parts that they then might understand.

6. Explain the concept of HAART, and how it is a combination of these groups. Explain that it is one of the best current treatments we have for HIV/AIDS, but is not a cure. Individuals who stay on this treatment plan will live longer\(^{15}\), but more studies need to be conducted to see how much longer the individuals live. Use this as an intro to HIV-1 protease (Part 2 of “A Mutant in Our Midst”).

**ASSESSMENT SUGGESTIONS:**

- The two student handouts should be collected. These worksheets should have ensured that students:
  1. Understand the drug development process
  2. Understand how the different classes of HIV/AIDS drugs work

**Florida Next Generation Sunshine State Standards (NGSSS):**

1. Evaluated the impact of biotechnology on the individual, society and the environment, including medical and ethical issues by understanding the drug discovery process.

**EXTENSIONS:**

- Students can investigate individual HIV/AIDS drugs at [http://www.thebody.com/content/art40488.html](http://www.thebody.com/content/art40488.html). Reports or posters could be created to show general information about the drug, explain how it works, how much it costs, and what drug group it falls into (see groups above).

**RESOURCES/REFERENCES:**

- [http://aids.emedtv.com/hiv/hiv-medications.html](http://aids.emedtv.com/hiv/hiv-medications.html)
<table>
<thead>
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</tr>
<tr>
<td>Submitted to Food and Drug Administration (FDA) as an Investigational New Drug (IND)</td>
<td>FDA will determine if it is safe to be tested in humans.</td>
</tr>
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</tbody>
</table>
Name: __________________________________

Figure 1. Molecule structure of Saquinavir, one of the early drugs on the market that inhibited HIV.

1. How long do you think the following stages took to develop drugs similar to this?
   - Drug discovery?
   - Clinical trials?
   - Submission for Market Approval
2. What do you think the total time of drug discovery/development is?

3. How much do you think that the entire process costs?

4. When first approved in 1995, Roche (the pharmaceutical company who made the drug) introduced it under the name “Invirase”, which was a hard gel capsule. In 1997, Roche sold the drug as “Fortovase, which was a soft gel capsule. Hypothesize a reason as to why they needed to make this change.
Name: ______________

Figure 1. Molecule structure of Saquinavir, one of the early drugs on the market that inhibited HIV.

1. How long do you think the following stages took to develop drugs similar to this?
   - Drug discovery?
     3-7 years
   - Clinical trials?
     6-7 years
   - Submission for Market Approval
     6 months to 2 years

2. What do you think the total time of drug discovery/development is?
   About 10-15 years
3. How much do you think that the entire process costs?

*Depending on the source, the acceptable range is $55 million to $2 billion*

4. When first approved in 1995, Roche (the pharmaceutical company who made the drug) introduced it under the name “Invirase”, which was a hard gel capsule. In 1997, Roche sold the drug as “Fortovase, which was a soft gel capsule. Hypothesize a reason as to why they needed to make this change.

*Although it moved through all of the stages of drug development and discovery, the hard pill was not readily absorbed, and led to viral resistance in some of the first individuals who took it.*
STUDENT PAGES: HIV MEDICATION TYPES – STUDENT HANDOUT

Name: __________________________

INSTRUCTIONS: Based on the group names, work in groups of 3 to deduce what these drug groups may do.

Nucleoside/Nucleotide Reverse Transcriptase Inhibitors-

Non-Nucleoside Reverse Transcriptase Inhibitors-

Protease Inhibitors-

Fusion or Entry Inhibitors-

Integrase Inhibitors-
Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTI) -

NRTIs interfere with the action of an HIV protein called reverse transcriptase. The NRTI is a molecule similar to the nucleotides normally found in DNA, but when they are added to the DNA strand being synthesized (from the viral RNA), the DNA replication process terminates.

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) -

NNRTIs also stop HIV from replicating within cells by directly inhibiting the reverse transcriptase protein.

Protease Inhibitors (PI) -

PIs inhibit protease, which is protein involved in the normal HIV replication process. Protease cleaves (cuts) a viral polypeptide to create some of the necessary proteins in HIV.

Fusion or Entry Inhibitors -

Fusion or entry inhibitors prevent HIV from binding to or entering human immune cells.

Integrase Inhibitors -

Integrase inhibitors interfere with the integrase enzyme, which HIV needs to insert its genetic material into the human’s DNA.
LESSON FOUR: “A MUTANT IN OUR MIDST, PART 2”

KEY QUESTION(S): How are drugs developed if HIV mutates so quickly?

KEY SCIENCE CONCEPTS: HIV, AIDS, drug development, bacterial transformation, HIV-1 Protease, mutations

OVERALL TIME ESTIMATE: 60 Minutes of prep, 90 minutes for activity (60 the first day, 30 the following)

LEARNING STYLES: Use of technology, kinesthetic

VOCABULARY:
Ampicillin - An antibiotic that is semi-synthetic and is closely related to penicillin.
Competency - A state that a bacterial cell has to be in, in order to accept a plasmid.
Conformation - The proper 3-dimensional shape that a protein takes. This shape will determine its function.
Polypeptide - A polymer (chain) of amino acids
Primary sequence - The order of amino acids that make up the protein
Selective media - Growth media that will not allow certain bacteria to grow, but will allow others (with a specific trait) to grow.
Transformation - The insertion of a plasmid into bacterial cells.
Virion - Virus particle.

LESSON SUMMARY: In “A Mutant in Our Midst, Part 2”, students will simulate the first steps of HIV-1 protease research by transforming bacteria with fluorescent proteins and cloning genes. A focus on HIV-1 protease will be used as an example target for medications.

STUDENT LEARNING OBJECTIVES:
Student will be able to...
1. Complete the process of bacterial transformation.
2. Successfully model how changes in a genotype can impact an organism’s phenotype.
3. Understand the function of HIV-1 protease and how it is inhibited.

Florida Next Generation Sunshine State Standards (NGSSS) for “Part 1” and “Part 2”:
SC.912.L.16.10
SC.912.L.18.4

Advanced Placement (AP) Biology Learning Outcomes “Part 1” and “Part 2”:
LO 4.1
LO 4.2
LO 4.3
LO 4.9
MATERIALS:

- **ESSENTIAL:**
  - Bacterial transformation materials required (if using the recommended Edvotek kit- “Transformation of E. coli with Blue and Green Fluorescent Proteins”)
    - Automatic Micropipette (5-50 μl)
    - Disposable micropipette tips
    - Two Water baths (37°C and 42°C)
    - Thermometers
    - Incubation Oven (34°C and 37°C)
    - Pipet pumps or bulbs
    - Ice
    - Marking pens
    - Bunsen burner, hot plate or microwave oven
    - Hot gloves
    - Long wave U.V. light
    - 10 Serological disposable pipettes (10 x 0.1 mL)

BACKGROUND INFORMATION: Protease inhibitors are a promising group of drugs. This lesson (and following lessons) takes a closer look at how some of these medications are developed. HIV-1 Protease is an enzyme that is normally found in HIV and is vital to its successful reproduction. This enzyme cleaves a polypeptide inside of a newly formed (inactive) virion. These cleaved, smaller polypeptides take their normal conformation, and the virion is now active and infectious. Because of this, scientists have researched the molecular structure in order to understand how to inhibit it. It has been successfully inhibited by multiple drugs on the market today (all HIV-1 protease inhibitors). However, because of the fast mutation rate of HIV, there are now drug resistant strains. These strains have minor changes in their primary (protein) sequence, but can render these same drugs ineffective. Inhibition of these mutant forms is currently under investigation by many individuals, including Professor Ben Dunn (and his laboratory) at the University of Florida, College of Medicine. One of the most efficient ways to obtain these proteins is by inserting the gene that codes for HIV-1 protease into bacteria via transformation. The HIV-1 protease gene and protein product are harmless to work with, but students will be completing a simulation instead, using genes that express fluorescent proteins to mimic HIV-1 protease.

Bacterial transformation is the process in which a gene of interest is inserted into bacteria. The bacteria can then express that new protein. The most common type of transformation done in the classroom is the insertion of a plasmid offering ampicillin resistance along with another selection factor such as blue/white screening or fluoresces. In order to transform the Escherichia coli, the cells must be in a state of competency. The simplest method of transforming E. coli is to use a combination of heat and calcium chloride. Although the effectiveness of transformation can be studied (see “extensions”), it can be stated that most of the E. coli will not be transformed. Because of this, selective media can be used to kill any of the bacteria that are not resistant to ampicillin. This should ensure that most of the bacteria growing on the plate have successfully been transformed.

The easiest way to obtain most of the materials needed for the transformation is to purchase a classroom-ready kit. One of the best kits for this lesson is made by Edvotek (“Transformation of E. coli with Blue and Green Fluorescent Proteins”, and can be found at http://www.edvotek.com/222). Although other kits that
have only one fluorescing protein are available, many of the questions in this lesson require that there are two differently fluorescing proteins on the petri dish.

ADVANCE PREPARATION:

1. Prepare for the bacterial transformation lab according to kit instructions

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. (7 minutes) HIV-1 Protease background (short lecture and videos).
   a. Outline of activity:
      1. Introduce HIV-1 protease
      2. Watch the videos and show high-quality model of the molecule (links below)
      3. Introduce cloning
   b. Details of activity:
      Introduce the concept that the inhibition of HIV-1 protease is being heavily researched as a way to slow down HIV. There are two videos which do a great job of showing the role and structure of HIV-1 protease, and both of them should be shown to the class.
      Great video showing the overall function of HIV-1 protease:
      http://www.dnatube.com/video/12230/Protease-inhibitors-animation
      Video showing the structure of HIV-1 protease and how it is inhibited but good. Video is lower quality, but still worth watching:
      http://www.dnatube.com/video/1282/HIV-Protease
      After these videos have been shown, log into the Protein Data Bank and show students a higher-quality view of HIV-1 protease. Go to:
      After students have a general understanding of HIV-1 protease, tell students that they will be completing a large project that will investigate the effectiveness of four potential drugs in the inhibition of HIV-1 protease (if you plan on completing Lessons 4 and 5 of this unit). In order to investigate the effectiveness of these drugs, we will need lots of copies of this protein. The best way to obtain these proteins is by inserting the gene (that creates HIV-1 protease) into bacteria via transformation. The HIV-1 protease gene and protein product are harmless to work with, but they will be completing a simulation instead.

2. (90 minutes: 60 minutes DAY 1, 30 minutes DAY 2) Bacterial transformation/cloning simulation

IMPORTANT NOTE - ACTIVITY INCLUDES ANTIBIOTICS AND SHOULD NOT BE COMPLETED BY STUDENTS WHO HAVE ALLERGIES TO AMPICILLIN, PENICILLIN, TETRACYCLINE, OR KANAMYCIN.
Also assure students that the material they are working with is not an infectious part of HIV-1, but instruct student to be careful working with the bacteria (follow all recommended safety guidelines in the transformation kit).
For this activity two differently fluorescing proteins (FPS) will be used (blue and green). These FPS are meant to simulate the HIV-1 protease gene- both the wild type and a mutant type. The green FP will
simulate the wild type HIV-1 protease and the blue FP will simulate the mutant HIV-1 protease. Do not tell students they will be using two different plasmids and will be getting two differently colored colonies. The blue FP and green FP are both very similar in structure, just as HIV-1 wild type is very similar in structure to the HIV-1 mutant types.

To setup for this activity, follow the procedures in the purchased kit. However, you will mix the green FP and the blue FP together and label as “HIV-1 Protease Plasmid.” A 3:1 ratio of green FP to blue FP is recommended, so more colonies are green and appear to be the wild type. Additionally, make sure to change any named plasmids in student instructions to “HIV-1 Protease Plasmid.”

ASSESSMENT SUGGESTIONS:

- The questions associated with bacterial transformation should be collected. These questions should have ensured that students:

Florida Next Generation Sunshine State Standards (NGSSS):
1. Related the structure and function of enzymes (HIV-1 protease).

Advanced Placement (AP) Biology Learning Outcomes:
2. Explain the connection between the sequence and the subcomponents of a biological polymer and its properties by explaining how a mutated HIV-1 protease might behave differently.
3. Refine representations and models to explain how the subcomponents of a biological polymer and their sequence determine the properties of that polymer by creating different sequences of DNA that have the same and different products.
4. Use models to predict and justify that changes in the subcomponents of a biological polymer affect the functionality of the molecule by creating different sequences of DNA that have the same and different products.
5. Predict the effects of a change in a component(s) of a biological system on the functionality of HIV.
6. Use representations and models to communicate scientific phenomena and solve scientific problems by creating different sequences of DNA that have the same and different products.
7. Engage in scientific questioning to extend thinking or to guide investigations within the context of the AP course by questioning why there are two differently fluorescing products on their petri dishes.
8. Perform data analysis and evaluation of evidence by hypothesizing why there are two differently fluorescing products on their petri dishes.

EXTENSIONS:

- The recommended bacterial transformation kit has excellent extensions, including the determination of transformation efficiency. Additionally, BioRad has a SDS-PAGE extension that allows for the separation of the GFP (http://www.bio-rad.com/prd/en/US/LSE/PDP/a41608e9-b348-43e0-98bb-d0ae12664e06/pGLO20SDS-PAGE20Extension).

RESOURCES/REFERENCES:
Background: As we have already learned, the inhibition of HIV-1 protease is an important method to slow down the HIV infection. In order to study HIV-1 protease, scientists must produce large quantities of it. This is accomplished through bacterial transformation using Escherichia coli and a plasmid. In this activity, you will be inserting a plasmid that contains the genes for ampicillin resistance and HIV-1 protease which (in this simulation) glows in the proper growth conditions. If glowing colonies are observed under the proper conditions, then it can be assumed that the transformation was successful, and a large amount of HIV-1 protease was produced.

Methodology: See kit instructions

Questions:
1. Why did the control not grow on the plate with ampicillin? Why is this control important?

2. There is a chance that there will be multiple different colors of fluorescence on your plate. Why do you think this is (if there is only one color, find a group that has multiple colors)?

3. If the HIV-1 protease is different colors, suggest a hypothesis for how the protein changed:

4. What effect could this change to HIV-1 protease have on a HIV-1 protease inhibitor? Why?

5. If HIV-1 protease mutated, how would it affect HIV and the treatment of it?

6. Create two different models of DNA (by writing the nucleotide sequences). The first model should compare two strands of DNA that are different by 4 nucleotides, but still express the same protein. The second model should compare two strands of DNA that are different by only one nucleotide but would express a different protein (you will need the codon chart available in your textbook or online).
<table>
<thead>
<tr>
<th>DNA strand 1</th>
<th>DNA strand 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA =</td>
<td>mRNA =</td>
</tr>
<tr>
<td>AAs =</td>
<td>AAs =</td>
</tr>
</tbody>
</table>

7. Would the proteins that you created above behave the same or differently?

   Model #1 (the same or differently)? Why?

   Model #2 (the same or differently)? Why?
Questions:

1. Why did the control not grow on the plate with ampicillin? Why is this control important?
   The bacteria did not have the plasmid that encoded for a protein that offers ampicillin resistance. Because of the presence of ampicillin, the bacteria were not able to grow. The control is important because it allows scientists to compare how the change in the independent variable affects the dependent variable.

2. There is a chance that there will be multiple different colors of fluorescence on your plate. Why do you think this is (if there is only one color, find a group that has multiple colors)? Although, as the instructor, you know that they used two different plasmids that express two differently fluorescing proteins, students do not. Their answers should reflect that the gene most likely mutated and is now producing a different product.
   Interesting note: Although this is a simulation, there are two amino acid substitutions which cause the fluorescence difference, and this mimics the same force at work when looking at the HIV-1 wild type versus some mutant types. The change is caused by a substitution of only a couple amino acids, and this change can cause major differences.

3. If the HIV-1 protease is different colors, suggest a hypothesis for how the protein changed:
   The DNA/plasmid mutated possibly because of a substitution, insertion, or deletion.

4. What effect could this change to HIV-1 protease have on a HIV-1 protease inhibitor? Why?
   The inhibitor would act differently. Most likely, the inhibitor would not work as efficiently or it might not work at all.

5. If HIV-1 protease mutated, how would it affect HIV and the treatment of it?
   HIV would most likely BEHAVE similarly, but it would possibly be more difficult to treat an individual who is infected with the HIV that has this mutated protease with currently available HAART treatments.

6. Create two different models of DNA (by writing the nucleotides). The first model should compare two strands of DNA that are different by 4 nucleotides, but still express the same protein. The second model should compare two strands of DNA that are different by only one nucleotide but would express a different protein (you will need the codon chart available in your textbook or online).
   There are many different possible answers for this question. Below is an example:

<table>
<thead>
<tr>
<th>Model #1 (four differences, same protein)</th>
<th>Model #2 (one difference, different protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA strand 1 = AAG GGC GGC GGG</td>
<td>DNA strand 1 = AAC GGC GGC GGG</td>
</tr>
<tr>
<td>mRNA = UUC CCG CCG CCC</td>
<td>mRNA = UUG CCG CCG CCC</td>
</tr>
</tbody>
</table>
AAs = phe pro pro pro  

AAs = leu pro pro pro  

DNA strand 2 = AAA GGG GGG GGC  

DNA strand 2 = AAA GGC GGC GGG  

mRNA = UUU CCC CCC CCG  

mRNA = UUG CCG CCG CCC  

AAs = phe pro pro pro  

AAs = phe pro pro pro  

7. Would the proteins that you created above behave the same or differently?

Model #1 (the same or differently)? Why?

_The same. It is the same protein._

Model #2 (the same or differently)? Why?

_Differently, it is a different protein._
LESSON FIVE: “OPTIMIZE AND INHIBIT – AND KEEP YOUR JOB”

KEY QUESTION(S): How do enzymes work and how can I optimize a reaction? How can an inhibitor slow down the optimized reaction?

KEY SCIENCE CONCEPTS: Enzymes, activation energy, IC\textsubscript{50}, LD\textsubscript{50}, enzyme inhibition, drug development/discovery, spectrophotometry.

OVERALL TIME ESTIMATE: 3 days

LEARNING STYLES: Kinesthetic

VOCABULARY:
Competitive inhibitor - The inhibitor “competes” with the normal substrate for the active site.

Crystallization - An organized structure of purified molecules which align themselves in repeating units, forming the crystal. A pure protein crystal can then be analyzed using x-ray crystallography to determine the tertiary or 3-D structure of the molecule of interest.

Denature - When a protein's conformation changes. If this protein is an enzyme, this will usually render it useless.

Enzyme - A molecule found in biological systems that speeds up a reaction by reducing the activation energy.

Horseradish peroxidase - A common enzyme that catalyzes a variety of reactions.

IC\textsubscript{50} - The amount/concentration required to inhibit the reaction by 50%

Inhibitor - A molecule that will slow down an enzymatic reaction.

LD\textsubscript{50} - Median lethal dose, or the dose that will kill 50% of the tested population.

Lock and key mechanism - The first model used to explain how an enzyme and substrate interacted. It was meant to show the action of the substrate entering the enzyme is extremely specific.

Non-competitive inhibitor - The inhibitor fits into a different site from the active site, but when doing so, changes the normal conformation of the active site, which prohibits the normal substrate from entering.

o-Dianisidine - A dye that, when oxidized, turns from clear to brown.

Substrate - The molecule that the enzyme acts upon.

X-ray crystallography - The use of x-rays to determine the atomic structure of a crystallized molecule.

LESSON SUMMARY: Students will optimize enzyme kinetics of simulated HIV-1 protease. Additionally, students will work to inhibit an enzymatic reaction (simulated HIV-1 protease) by the manipulation of different inhibitors in order to find the most effective product.
STUDENT LEARNING OBJECTIVES:
The student will be able to...
1. Students are able to optimize enzymatic activity by adjusting variables (pH) through the use of data analysis.
2. Students are able to determine the most effective way to inhibit an enzyme through the use of data analysis.
3. Students are able to successfully describe the interactions that an enzyme will have with a substrate or an inhibitor.

Florida Next Generation Sunshine State Standards (NGSSS) that are addressed:
SC.912.L.16.10
SC.912.L.18.11

Advanced Placement (AP) Biology Learning Outcomes that are addressed:
LO 4.17
Science Practice 1
Science Practice 2
Science Practice 3
Science Practice 4
Science Practice 5
Science Practice 6

Advanced International Certificate of Education (AICE) Biology Learning Outcomes that are addressed:
C (b).
C (c).
C (d).
C (e).

MATERIALS:

- **ESSENTIAL:**
  - Computers with attached spectrophotometer (such as the SpectroVis Plus Spectrophotometer from Vernier or the USB650 Red Tide Spectrometer from Ocean Optics)
  - Cuvettes for spectrophotometer
  - Pipettes (five P20, five P200, five P1000)
  - Disposable micropipette tips
  - Microcentrifuge tubes (1.5ml, 20 per group of students)
  - 50 Cetrifuge tubes (50.0mL. For containing larger amounts that will not fit in microcentrifuge tubes-such as stock solutions)
  - Chemicals (Table 1)
  - Distilled Water (2L should be more than adequate, or a gallon from local grocer)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Sigma-Aldrich #</th>
<th>Price</th>
<th>Description</th>
</tr>
</thead>
</table>
Table 1. Needed chemicals with associated information.

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Stock No.</th>
<th>Price</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>o-Dianisidine dihydrochloride</td>
<td>D3252-25G</td>
<td>$174.50</td>
<td>Substrate 1 in reaction (enough for 125 trials)</td>
</tr>
<tr>
<td>4-Aminobenzoic Acid</td>
<td>A9878-25G</td>
<td>$21.20</td>
<td>Enzyme inhibitor</td>
</tr>
<tr>
<td>Horse Radish Peroxidase (HRP)</td>
<td>P8125-25KU</td>
<td>$83.40</td>
<td>Enzyme</td>
</tr>
<tr>
<td>3% Hydrogen Peroxide</td>
<td>(local drug store)</td>
<td>$6.00 (approx.)</td>
<td>Substrate 2 in reaction</td>
</tr>
<tr>
<td>Tris-EDTA buffer, pH 8.0</td>
<td>93283-100ML</td>
<td>$14.80</td>
<td>Buffer</td>
</tr>
<tr>
<td>Tris-EDTA buffer, pH 7.4</td>
<td>93302-100ML</td>
<td>$14.50</td>
<td>Buffer</td>
</tr>
</tbody>
</table>

**BACKGROUND INFORMATION:** A foundational concept in the biological sciences is the understanding of how an enzyme works. Enzymes have been known for over one hundred years,¹ and in 1894 Fisher suggested the "lock and key" mechanism of enzymes and the molecules they interact with (the substrate).² This interaction always includes the reduction of the activation energy—which is the amount of energy required to begin a chemical reaction. Soon after, Leonor Michaelis and Maud Leonora Menen came up with a representation of what takes place in a standard enzymatic reaction:

\[
E + S \rightarrow ES \rightarrow E + \text{products}
\]

where "E" is the enzyme, "S" is the substrate, and "ES" is the enzyme-substrate complex. This model is still widely accepted, and will be the assumed pathway for this lesson (not including the addition of an inhibitor). In 1926 James Sumner showed that enzymes were proteins⁴⁻⁵ which was then confirmed by Northrop and Stanley in 1930 via crystallization.⁵ Not much was known about a protein’s 3-dimensional structure until 1965 when David Phillips published a report indicating actual structure of lysozyme through x-ray crystallography.⁶ To this day, protein structures are studied through x-ray crystallography in order to understand their atomic structure. The “lock and key” description developed in 1894 by Fischer was modified in 1958 by Koshland. Koshland hypothesized “the induced fit model” which stated that the enzyme changed conformation when interacting with the substrate.⁷ This model is still used today to explain how an enzyme interacts with a substrate.

Many enzymes can be inhibited through different mechanisms. The two most common groups of inhibitors are and non-competitive. There are different environmental factors that can slow down or speed up the actions of an enzyme. Temperature, pH, and substrate/enzyme concentrations are the most commonly studied of these factors. Each enzyme will have a temperature and pH in which it ideally works (achieves its maximum rate of reaction). If the enzyme is exposed to extremes of these factors, there is a chance the enzyme will denature.
Enzyme kinetics is the examination of the enzymatic reaction, which is commonly done by looking at the rate of reaction. One of the most accurate ways to investigate the rate of reaction is by the use of spectrophotometry. Spectrophotometers measure the absorbance of light in a sample and can be used to accurately track an enzymatic reaction. As the reaction proceeds, the data is stored in a computer for further analysis. Entry of data into software, such as Microsoft Excel allows the graphic representation of this data.

Figure 1. The relation between substrate concentration and reaction rate. From http://en.wikipedia.org/wiki/File:Michaelis-Menten_saturation_curve_of_an_enzyme_reaction.svg.
HIV-1 protease inhibition is commonly studied with spectrophotometry. Although it is safe to work with HIV-1 protease, the color change of the sample is entirely too small to be detected by spectrophotometers used in classrooms. Because of this, a simulation must be used to mimic the reaction. Any enzymatic reaction that includes a product that is a different color than the start of the reaction will simulate this nicely. For this activity, the oxidation of *o-Dianisidine* (via H$_2$O$_2$) with the enzyme *horseradish peroxidase* (HRP) is used. The reactants are all clear, and the product of the enzymatic reaction is brown. This color change can be recorded in real-time by the use of a common classroom spectrophotometer interfaced with a computer.

**ADVANCE PREPARATION:**

*Inform students the DAY BEFORE the activity to have close toed shoes, long sleeves, and long pants on for this lab.*

There is a good deal of preparation for this activity. In order to make the stock solutions needed for all of the activities within this lesson, please complete the following steps. Students should be working in groups of 4. The limiting reagent will most likely be o-Dianisidine, and the quantity recommended above will allow for 125 enzymatic trials. Adjust quantities as needed:

1. Creation of Inhibitors (Potential "drugs". Put 25.0mL in each in 50.0mL centrifuge tubes-one per group-and label as the following):
   a. "Drug 1": 0.06g/10mL 4-Aminobenzoic Acid in water ->44-mM conc.
   b. "Drug 2": 0.03g/10mL 4-Aminobenzoic Acid in water ->22-mM conc.
   c. "Drug 3": Distilled water (not an inhibitor, but still label as "drug 3". It should have minimal, if any, impact on the reaction).
   d. "Drug 4": 0.12g/10mL 4-Aminobenzoic Acid in water ->88-mM conc.
2. **o-Dianisidine Stock**: add **25-mL** water to **25mg** bottle of o-Dianisidine to make **1mg/mL** stock (distribute 5.0mL into 50.0mL centrifuge tubes and label as “o-Dianisidine”).
   a. Note: Prepare this solution in a fume hood, with gloves, goggles, and proper safety barriers in place.
3. **Horseradish Peroxidase (HRP) Stock**: add 50ml of pH 7.4 buffer to 25mg HPR bottle to make ~500U/mL stock (distribute 10.0mL into 50.0mL centrifuge tubes and label as HIV-1 Protease).
4. Put 40.0mL distilled water into five 50.0mL centrifuge tubes, and label as H2O.
5. Put 20.0mL of buffer (both pH 7.4 and 8.0) into 50mL centrifuge tubes.

**PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:**

1. (60 minutes) Baseline reaction optimization:
   - Each group of students should have:
     - Computer with spectrophotometer
     - 20.0mL of buffer (both pH 7.4 and 8.0)
     - 25.0mL of o-Dianisidine Stock
     - 10mL HRP
     - 40.0mL distilled water
     - 25 microcentrifuge tubes
     - 10 cuvettes
     - Pipettes (one of each)
     - Disposable micropipette tips

   Students working in groups of four will be looking at an enzymatic reaction in order to investigate the effectiveness of certain inhibitors (possible “drugs”). This simulates the exact process that is done in research laboratories that are investigating the inhibition of HIV-1 protease. The successful inhibitors are then further investigated as potential drugs. Students need to a solid understanding of general enzyme function and structure. The overall reaction that will take place in this simulation is as follows:

   \[
   \text{H}_2\text{O}_2 + \text{Reduced o-Dianisidine} \rightarrow \text{Peroxidase} \rightarrow \text{Oxidized o-Dianisidine}\n   \]

   (colorless) (brown)

   Students will use the spectrophotometer to track the kinetics of the reaction and then graph the data in Excel (or equivalent program). The optimization of the enzymatic reaction is an important step to establish a baseline reaction to inhibit. Although this step could be skipped, it allows students to become comfortable using the spectrophotometers and pipettes. Two different tris-EDTA buffers (one with a pH of 7.4 and the other a pH of 8.0) will be used. Allow students to try both buffers in the reaction, and determine which provides the optimal condition for the enzymatic reaction.

   **SAFETY NOTE**: Make sure students are wearing splash goggles and gloves at ALL times. Students should have close toed shoes, long sleeves, and long pants on.

2. (120 minutes, split between multiple days) Enzyme inhibition:
   - Each group of students should have:
     - Computer with spectrophotometer
     - 20.0mL of buffer (both pH 7.4 and 8.0)
     - 25.0mL of o-Dianisidine Stock
     - 10mL HRP
     - 40.0mL distilled water
     - 25.0mL of each “drug”
- 25 micocentrifuge tubes
- 10 cuvettes
- Pipettes (one of each)
- Disposable micropipette tips

Once students have optimized the reaction, the next step is to determine what the best inhibitor ("drug") is to use to slow down the reaction by 50%. Do not tell students what the inhibitors actually are, just label them as "Drug 1", "Drug 2", "Drug 3", and "Drug 4". It is very important to use the established LD_{50} values during this exercise; otherwise students will add large amounts of some of the "drugs" to the reaction. The goal of this activity is not to completely inhibit the enzyme, but to reduce the baseline reaction rate by half (find the IC_{50}). Students will have to use trial and error to find correct amounts of inhibitor to do this.

**ASSESSMENT SUGGESTIONS:**

- Students should turn in all questions included with the lesson. Make sure students save all Excel data for "Lesson 5".
- Students were able to optimize the enzymatic activity of HRP by adjusting variables (pH) through the use of data analysis.
- Students were able to determine the most effective way to inhibit HRP through the use of data analysis.
- Students were able to successfully describe the interactions that an enzyme will have with a substrate or an inhibitor.

**EXTENSIONS:**

- Different buffers (at different pH values) are available for the enzyme optimization step. Adding more variety with the buffers can allow for more trial and error, but more materials will be required.
- Enzyme concentration can be varied to observe the effects of enzyme concentration versus substrate concentration.

**RESOURCES/REFERENCES:**

- Kühne coined the word "enzyme" in: W. Kühne (1877) "Über das Verhalten verschiedener organisirter und sog. ungeformter Fermente" (On the behavior of various organized and so-called unformed ferments), Verhandlungen des naturhistorisch-medicinischen Vereins zu Heidelberg, new series, vol. 1, no. 3, pages 190–193. The relevant passage occurs on page 190: "Um Missverständnissen vorzubeugen und lästige Umschreibungen zu vermeiden schlägt Vortragender vor, die ungeformten oder nicht organisirten Fermente, deren Wirkung ohne Anwesenheit von Organismen und ausserhalb derselben erfolgen kann, als Enzyme zu bezeichnen." (Translation: In order to obviate misunderstandings and avoid cumbersome periphrases, [the author, a university lecturer] suggests designating as "enzymes" the unformed or not organized ferments, whose action can occur without the presence of organisms and outside of the same.)
The optimization of an enzymatic reaction is an important step in drug discovery. The reaction should be at its optimal rate in order to study the effectiveness of lead compounds. The adjustment of certain factors, such as temperature, enzyme concentration, and pH all can impact the reaction rate. In this activity, you will find the optimal pH for HIV-1 protease (that was created in the previous lesson), with no inhibition. When HIV-1 protease assay and \( \text{H}_2\text{O}_2 \) are added together, the colorless solution will turn to a brown color (via the oxidation of the dye o-Dianisidine). The spectrophotometer needs to be set to measure “absorbance” at a wavelength of 445 nm.

**SAFETY NOTE:** Make sure to be wearing splash goggles and gloves at ALL times. Alert the instructor if any solutions are spilled.

**Methodology:**

1. Label a 1.5ml microfuge tube “HIV-1 protease assay reagent 1”
2. Prepare HIV-1 protease assay reagent in the tube by adding the following (volume always needs to equal 550ul once all solutions are added to this tube):
   a. 200uL water
   b. 200uL 0-Dianisidine stock
   c. 100uL HIV-1 protease stock
   d. 50uL pH 7.4 Tris-EDTA Buffer
3. Label a 1.5mL microfuge tube \( \text{H}_2\text{O}_2 \).
4. Prepare \( \text{H}_2\text{O}_2 \) solution by adding the following to “\( \text{H}_2\text{O}_2 \)” tube:
   a. **10-uL 3\% \( \text{H}_2\text{O}_2 \) in 30ml of water**
5. Add 500uL HIV-1 protease assay reagent to the cuvette while it is inside of the spectrophotometer.
6. Start data collection and simultaneously add **300uL \( \text{H}_2\text{O}_2 \)” to the cuvette.
7. Collect data for **2 minutes**.
8. Stop data collection. If the computer program has tools to determine rate, go ahead and determine rate for the first minute.
9. Export data to Excel for analysis, label, graph (see below), and then save (if the program does not have this tool, then use rate=\( \Delta \) [product]/ \( \Delta \) time).
10. Label a 1.5ml microfuge tube “HIV-1 protease assay reagent 2”
11. Prepare second HIV-1 protease assay reagent by adding the following to the labeled microfuge tube (volume always needs to equal 550ul):
    a. 200-uL water
    b. 200-uL 0-Dianisidine stock
    c. 100-uL HIV-1 protease stock
    d. 50-uL pH **8.0** Tris-EDTA Buffer
12. Add 500uL “HIV-1 protease assay reagent 2” to the cuvette while it is inside of the spectrophotometer.
13. Start data collection and simultaneously add 300uL \( \text{H}_2\text{O}_2 \) to the cuvette.
14. Collect data for **2 minutes**.
15. Stop data collection. If the computer program has tools to determine rate, go ahead and determine rate for the first minute.
16. Export data to Excel for analysis, label, graph (see below), and then save (if the program does not have this tool, then use rate=Δ [product]/ Δ time).

**Instructions to graph data in Microsoft Excel:**

1. Create two vertical columns.
2. Label the first one “Time”, and the second one “Absorbance”.
3. The data collected from the spectrophotometer should be available in the software used to run the spectrophotometer on the computer. Once the data has been found, copy the data (Time and Absorbance) to their respective columns in Excel.
4. Usually some “data trimming” is necessary (there was probably a fraction of a second, or a few seconds, between the time you began the run and when you put in the H₂O₂. Delete this unneeded data).
5. Highlight all of the data.
6. Create a scatter plot (click “insert”, then “graph”, then “scatter” or similar- depending on what version is being used).
7. You can right click on the graph to enter a title for the graph and titles to the axis.
1. What is the optimal pH for this enzymatic reaction? How do you know?

2. Create a trend line for your data. Right click data on graph, and then click “add trend line”. There are multiple options for trend lines, but choose “logarithmic”, and also check the box for “add R-squared value”, click “close”. The trend line and R^2 value should show up on the graph. The R^2 value is a fraction between 0.0 and 1.0 and tells you how well your line fits your data. The higher the R^2 value, the better the fit.

3. What are the R^2 values for both of your datasets? Are your lines a good fit for your data?
1. What is the optimal pH for this enzymatic reaction? How do you know?
   
   \[ \text{pH= 7.4, the reaction rate was faster.} \]

2. Create a trend line for your data. Right click data on graph, and then click “add trend line”. There are multiple options for trend lines, but choose “logarithmic”, and also check the box for “add R-squared value”, click “close”. The trend line and R\(^2\) value should show up on the graph. The R\(^2\) value is a fraction between 0.0 and 1.0 and tells you how well your line fits your data. The higher the R\(^2\) value, the better the fit.

3. What are the R\(^2\) values for both of your datasets? Are your lines a good fit for your data?
   
   The R\(^2\) values will change depending on the group. Usually, the lines are a fairly good fit to the data (the higher the better), but some data trimming might be necessary (look at the data to determine when the H\(_2\)O\(_2\) was actually added, and let that be the start of the data used in the graph).
Now that you have optimized the HIV-1 protease enzymatic reaction, you will need to find a drug that can inhibit the reaction. It is easy to understand that if you put enough inhibitor in the reaction it would most likely stop. However, these drug candidates each have a LD$_{50}$. The LD$_{50}$ stands for the “median lethal dose”, or the dose that will kill 50% of a tested population before 60 days. The unit that is typically used is mg/kg of body weight, but for the sake of this activity we will simplify. Each drugs LD$_{50}$ value will be given as a total amount allowed. Clearly, we want to find the drug that is least harmful for those taking it, while also inhibiting the reaction most effectively. A drug might inhibit a reaction fully, but if it kills half of those taking it, it would never make it past pre-clinical trials.

You are tasked to find a drug candidate, that when used in the proper concentration, will inhibit the reaction by 50% (IC$_{50}$, or the amount/concentration required to inhibit the reaction by 50%). This is done by finding a reaction rate that is half of your baseline. You have already established a baseline reaction rate (previous exercise). Find the correct concentration(s) of the potential drugs that will inhibit the reaction by 50% but will harm the fewest people (stays as far away from the LD$_{50}$ as possible). The spectrophotometer needs to be set to measure “absorbance” at a wavelength of 445 nm.

Released information about each inhibitor:

- **Drug #1** - 50uL of this drug will contain .50mg of active ingredient. The LD50 for this drug is .50mg/per person.
- **Drug #2** - 50uL of this drug will contain .10mg of active ingredient. The LD50 for this drug is .30mg/per person.
- **Drug #3** - 50uL of this drug will contain .25mg of active ingredient. The LD50 for this drug is 3.0mg/per person.
- **Drug #4** - 50uL of this drug will contain .50mg of active ingredient. The LD50 for this drug is 1.0mg/per person.

**Methodology**

1. Prepare HIV-1 protease inhibition assay reagents (volume always needs to equal 550ul) in a microcentrifuge tube (make sure to label):
   - “X” uL drug stock solution (you are allowed to change this amount, but keep in mind each drug’s LD50 value)
   - 200-uL 0-Dianisidine stock
   - 100-uL HIV-1 protease stock
   - 50-uL pH 7.4 Buffer
   - “Y” uL of water
NOTE: An easy way to think of this: X + Y + 400uL 0-Dianisidine + 100uL HIV-1 protease + 50uL Buffer = 550uL, where “X” is a variable amount of drug, and “Y” is a variable amount of water to bring the total volume to 550uL. If 200uL of a drug is used, no water is added.

2. If none remains from previous activity, prepare additional H₂O₂ solution:
   **10-uL 3% H₂O₂ in 30ml of water**
3. Add 500uL HIV-1 protease assay reagent to the cuvette while it is inside of the spectrophotometer.
4. Start data collection and simultaneously add 300uL H₂O₂ to the cuvette.
5. Collect data for **2 minutes**.
6. Stop data collection. If the computer program has tools to determine rate, go ahead and determine rate for the first minute.
7. Export data to Excel for analysis, label, graph (see below), and then save (if the program does not have this tool, then use rate=Δ [product]/ Δ time).
8. Repeat steps 1-6. Change the drugs and quantities being used in “Step 1”, but make sure to keep thorough records for each one (create one graph at a time, and label each one before moving on to the next trial).

NOTE: It will take trial and error to find out which drug is ideal- in fact there may be two drugs that are equivalent. This will take multiple trials.
1. Your boss wants to know your results for each drug. For each drug, explain your FINDINGS (not your conclusions).
   Drug #1

   Drug #2

   Drug #3

   Drug #4

2. What is your final recommendation? Which drug should continue to be investigated (remember, a wrong answer could cost your boss a HUGE amount of money, and could cost you your job...)?

3. What do you recommend to be done with the other drug candidates? Should they be abandoned?

4. Explain the interaction that the inhibitor has with the enzyme (explain both competitive and non-competitive)?
5. What is the role of HIV-1 protease in this reaction?
1. Your boss wants to know your results for each drug. For each drug, explain your FINDINGS (not your conclusions).
   Drug #1 - *I have no idea until I actually try this lab*...
   Drug #2 - *I have no idea until I actually try this lab*...
   Drug #3 - *This drug will not work to inhibit HIV-1 protease, even at its LD<sub>50</sub>. It is just water*...
   Drug #4 - *I have no idea until I actually try this lab*...

2. What is your final recommendation? Which drug should continue to be investigated (remember, a wrong answer could cost your boss a HUGE amount of money, and could cost you your job...)?
   *Not sure yet*...

3. What do you recommend to be done with the other drug candidates? Should they be abandoned?
   *It is possible that a medicinal chemist could change the molecule slightly to make it more effective (or less toxic), but there is little hope for “Drug 3”.*

4. Explain the interaction that the inhibitor has with the enzyme (explain both competitive and non-competitive)?
   *Competitive inhibitors “compete” with the normal substrate for the active site and non-competitive inhibitors fit into a different site from the active site, but when doing do, changes the normal conformation of the active site, which prohibits the normal substrate from entering.*

5. What is the role of HIV-1 protease in this reaction?
   *It is the enzyme that facilitates the reaction by lowering the activation energy.*
LESSON SIX: “THE PERFECT CRYSTAL”

KEY QUESTION(S): What is done in the drug discovery process after a lead compound is identified through enzyme kinetics? How is protein crystallization completed? What is the purpose of crystallization? What is x-ray crystallography?

KEY SCIENCE CONCEPTS: Protein crystallization, x-ray crystallography, scientific presentation of data.

OVERALL TIME ESTIMATE: 2 days in class, 2 (or more) days of homework

LEARNING STYLES: Kinesthetic

VOCABULARY:
Crystallization - This process allows macromolecules (such as proteins) to form crystals. The macromolecule will be “locked” in place which allows further study.

Hanging drop vapor diffusion method - Method used to crystallize proteins in which the protein is suspended above a well of solution (see below for methodology).

Hemoglobin - Protein that is found in red blood cells and shuttles O₂ and CO₂ around the body.

Sitting drop vapor diffusion method - Method used to crystallize proteins in which the protein is sitting above a well of solution (see below for methodology).

LESSON SUMMARY: Students will understand how, and why, proteins are crystallized using the crystallization of lysozyme as an example protein. Students will then take their data (from lessons 3, 4 and 5) and create a poster that shows what they did in the drug discovery/development process.

STUDENT LEARNING OBJECTIVES:
The student will be able to...
1. Understand how macromolecules are studied in modern laboratories.
2. Produce a product that is synonymous with a standard scientific publication.

STANDARDS
Florida Next Generation Sunshine State Standards (NGSSS):
SC.912.L.16.10:

Advanced Placement (AP) Biology Learning Outcomes:
LO 4.3
Science Practice 1
Science Practice 3
Science Practice 4
Science Practice 5
Science Practice 6
MATERIALS:

- **ESSENTIAL** (amount of material is adequate for 90 students, assuming groups of two and class size of 30 students):
  - Material from Hampton Research (see Table 1)
    - Pipettes (five P20, five P200, five P1000)
  - Disposable micropipette tips
  - 40 Microcentrifuge tubes (1.5ml. For stock solutions)
  - 10 Centrifuge tubes (50.0mL. For containing larger amounts that will not fit in microcentrifuge tubes- such as stock solutions)
  - 40 Disposable plastic pipettes (one per group)
  - Poster boards
  - NaCl (dry powder)
  - ddH₂O

<table>
<thead>
<tr>
<th>Material</th>
<th>Hamp. Res. Cat #</th>
<th>Price (per)</th>
<th>Quantity needed/comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethylene Glycol 50%, 6K</td>
<td>HR2-533</td>
<td>$89.00</td>
<td>2 needed.</td>
</tr>
<tr>
<td>1M Sodium Acetate, pH 4.5</td>
<td>HR2-789</td>
<td>$33.00</td>
<td>1 needed. Dilute to 0.5 M with distilled H₂O.</td>
</tr>
<tr>
<td>Lysozyme Kit</td>
<td>HR7-108</td>
<td>$66.00</td>
<td>1 needed.</td>
</tr>
<tr>
<td>Linbro Plates (case of 50)</td>
<td>HR3-110</td>
<td>$331.00</td>
<td>1 needed.</td>
</tr>
<tr>
<td>22mm Coverslips</td>
<td>HR3-221</td>
<td>$242.00</td>
<td>1 needed.</td>
</tr>
<tr>
<td>Microbridges</td>
<td>HR3-310</td>
<td>$72.00</td>
<td>2 needed.</td>
</tr>
<tr>
<td>Immersion Oil</td>
<td>HR3-613</td>
<td>$17.00</td>
<td>2 needed.</td>
</tr>
</tbody>
</table>

Table 1. Material from Hampton Research

- **SUPPLEMENTAL**: 3D structure of protease from 3D Molecular Designs

BACKGROUND INFORMATION: Macromolecule crystallization is a vital step in the understanding of atomic structures and interactions. Although this sounds like a very modern laboratory method, it has been around for over 160 years.1 In 1840, Hünefeld extracted blood from an earthworm, placed it between thin sheets of glass, and allowed it to dry very slowly resulting in crystallized hemoglobin.1 The basic principle that slow dehydration can create crystals was established. Individual scientists continued to crystallize hemoglobin, but it is reported that Funke was the first to publish a quality crystallization procedure.2 James Sumner (mentioned in Lesson 3) was the first to crystallize an enzyme.1,3,4 In the 1930s, scientists turned their attention to x-ray diffraction, which is a method in which x-rays are shot at the crystal, and a diffraction pattern is created. This diffraction pattern can then be interpreted, and atomic interactions can be studied. Different proteins require different conditions for ideal crystal growth, and there are still many proteins that have never been crystallized.

One of the most common methodologies for crystallization is the hanging drop vapor diffusion method, in which a drop of solution (with protein) is suspended above a concentrated solution that will vaporize. The crystal will then potentially form in the hanging drop, if the conditions are ideal. This method is economical and is a reasonably fast procedure to carry out. Another popular method is the sitting drop vapor diffusion method,
in which a drop of solution (with protein) is placed on a “bridge” above the well of solution. This method is easier to setup than the hanging drop. Both of these methods are commonly used in laboratories, but often the setup of the conditions is completely automated (and completed by a robotic arm). Each well represents a different condition.

It can be argued that protein (and macromolecule) crystallization/x-ray diffraction is the most important tool developed over the last 100 years to study biology. The structure of DNA was established by Watson and Crick, partially due to the x-ray diffraction image taken by Franklin in 1953 and countless drugs have been developed using this technology to understand atomic interactions. There is little doubt that the use of crystallization and x-ray diffraction is firmly established in biology, and will continue to be used well into the future.

The next step in drug discovery/development is the crystallization of the enzyme/inhibitor complex to determine the atomic interactions via x-ray crystallography. This activity is meant to simulate the process of HIV-1 protease/inhibitor crystallization by crystallizing the protein lysozyme (activity is based on “Crystallization Hands On, Lysozyme Crystallization”). This crystallization process produces consistently good products (crystals), even if the student’s methodology is not perfect. There are different ideal crystallization conditions for different proteins, and this activity allows students to explore different crystallization conditions. The ultimate goal is for students to find the ideal condition(s). Students will then observe their crystals and record results (if possible, pictures should be taken and saved of the crystals).

Students will then create a poster with their results from bacterial transformation, enzyme kinetics, and crystallization. The acceptable format can be changed, but see below (student instructions) for a general poster outline. The instructor needs to focus on the fact that it should look professional. It would be a good idea to show the class a research poster (either a poster already in your possession or from an image search of “scientific poster”). Possibly do not allow any hand written results (for ease, have students type the work up, print it on computer paper, and paste it to the poster boards). These posters should be hung in the halls of the school when completed.

ADVANCE PREPARATION:

1. All solutions need to be made the day before the activity, except lysozyme which should be made the day of the crystallization procedure. Methodology for this can be found in Table 2.

<table>
<thead>
<tr>
<th>Chemical/Solution</th>
<th>Assigned tube name</th>
<th>Volume per tube</th>
<th>Quantity of tubes needed</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>4M* NaCl</td>
<td>“A”</td>
<td>45.0mL</td>
<td>5</td>
<td>*Prepare this by putting 233.7g of NaCl into a beaker, and fill to 1L</td>
</tr>
<tr>
<td>0.5M* Sodium Acetate, pH 4.5</td>
<td>“B”</td>
<td>20.0mL</td>
<td>5</td>
<td>*Purchased solution was 1.0M. Dilute to 0.5M by adding 100mL ddH₂O before distributing.</td>
</tr>
<tr>
<td>50% PEG 6K</td>
<td>“C”</td>
<td>40.0mL</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>ddH₂O</td>
<td>“D”</td>
<td>50.0mL</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Lysozyme*</td>
<td>“E”</td>
<td>1000uL</td>
<td>5</td>
<td>Reconstitute by following manufactures guidelines</td>
</tr>
<tr>
<td>Immersion Oil</td>
<td>Immersion Oil</td>
<td>5.0mL</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
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<td>------</td>
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<td></td>
</tr>
</tbody>
</table>

Table 2. Methodology for setup.

**PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:**

1. (2 days) Protein Crystallization - (1 day for setup, 1 day for observation of results. Additionally, allow for 2 days or more of homework for poster creation. It would be ideal to explain the activity and distribute methodology the day before so there is adequate time to set up crystallization screens).
   a. Each group of 2 students should have:
      i. 1 Linbro Plate
      ii. Cover Slips (around 20)
      iii. Micro-bridges (10)
      iv. 1 Immersion oil and disposable plastic pipette

**ASSESSMENT SUGGESTIONS:**

- Students were able to understand how macromolecules are studied in modern laboratories by finding ideal conditions for protein crystallization.
- Students were able to produce a poster that is synonymous with standard scientific publication.

**EXTENSIONS:**

- Instead of posters, lab reports could be created. The format does not change from what is above, it is just a different product.
- Other proteins can possibly be crystallized. Students could attempt to crystallize the HRP from the previous lab under the ideal crystallization conditions of lysozyme and see the ideal conditions are not the same (it is unknown if HRP will crystallize under any of the above conditions).

**RESOURCES/REFERENCES:**

- The chemical nature of enzymes (Nobel lecture) See p.117: "It gave tests for protein and possessed a very high urease activity."
The crystallization of proteins and other macromolecules is necessary to determine the atomic interactions and 3-dimenional shape. In the last lesson, you found an ideal inhibitor for HIV-1 protease. In this lesson, you will be crystallizing the HIV-1 protease with the inhibitor in the active site. This crystal will then be shot with x-rays to produce an x-ray diffraction (see Figure 1). Although to the untrained eye this does not look like much, it shows scientists how the x-rays bounced off of the molecule. A trained individual can interpret this data (with the aid of software) to determine a 3-dimenional structure (see Figure 2). Determining this 3-dimenional structure can then allow scientists to understand the interaction, and then possibly modify the inhibitor to make it work better.


Figure 2. 3-dimenional cartoon model of HIV-1 protease with inhibitor (from http://people.mbi.ucla.edu/yeates/153AH_2009_project/sriphanlop.html)

One of the difficult parts about protein crystallization is that there is no “one set way” to crystallize a protein. This field of science is trial and error- try different conditions until a crystal grows. In this activity you will be given different parameters to try in order to find the ideal conditions to grow a crystal. The crystals will be small, but are easily visible under a dissecting microscope.

Solutions used in this procedure:

- Solution “A”- 4M NaCl.
- Solution “B”- 0.5M Sodium Acetate, pH 4.5
- Solution “C”- 50% PEG 6K
- Solution “D”- ddH₂O
- Solution “E”- HIV-1 protease with inhibitor in active site
- “Immersion oil”- used to make the individual wells “air-tight”

NOTES:
- The volume of each well after the addition of solutions should be 1mL.
- Mix the solutions in the wells thoroughly with your pipette.
- The lysozyme droplet should be 10uL (5uL of lysozyme + 5uL of the reservoir solution).
- The 50% PEG 6K is extremely thick. Pipette slowly to ensure no bubbles.

**Lysozyme Crystallization Screening Conditions**
(Set up these wells BEFORE moving to “methodology”)

Line 1 - Volume (in uL) for Na Acetate, pH 4.5 (solution “B”)
Line 2 - Volume (in uL) for NaCl (solution “A”)
Line 3 - Volume (in uL) for PEG 6K (solution “C”)
Line 4 - Volume (in uL) for ddH$_2$O (“D”)

Top view of Linbro plate (gray indicates actual wells):

<table>
<thead>
<tr>
<th></th>
<th>0.4M NaCl</th>
<th>0.8M NaCl</th>
<th>1.0M NaCl</th>
<th>1.2M NaCl</th>
<th>1.6M NaCl</th>
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</thead>
<tbody>
<tr>
<td><strong>Hanging Drop</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Well A1”</td>
<td>100</td>
<td>200</td>
<td>500</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>100(uL Na Acetate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100(uL NaCl)</td>
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<tr>
<td>200(uL PEG 6K)</td>
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<td></td>
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<td></td>
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<tr>
<td>600(uL ddH$_2$O)</td>
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<td>“Well A3”</td>
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<td></td>
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<td>400</td>
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<tr>
<td>“Well A4”</td>
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<td>100</td>
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</tr>
<tr>
<td><strong>Sitting Drop</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>“Well B1”</td>
<td>100</td>
<td>200</td>
<td>500</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>100(uL Na Acetate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100(uL NaCl)</td>
<td></td>
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<tr>
<td>200(uL PEG 6K)</td>
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<td></td>
</tr>
<tr>
<td>600(uL ddH$_2$O)</td>
<td></td>
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</tr>
<tr>
<td>“Well B2”</td>
<td>100</td>
<td>200</td>
<td>500</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>“Well B3”</td>
<td>200</td>
<td>450</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Well B4”</td>
<td>200</td>
<td>400</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Well B5”</td>
<td>200</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hanging Drop</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Well C1”</td>
<td>100</td>
<td>200</td>
<td>500</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>100(uL Na Acetate)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100(uL NaCl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500(uL PEG 6K)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300(uL ddH$_2$O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Well C2”</td>
<td>100</td>
<td>250</td>
<td>500</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Well C3”</td>
<td>250</td>
<td>500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Well C4”</td>
<td>500</td>
<td>500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>“Well C5”</td>
<td>150</td>
<td>100</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
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</tr>
</tbody>
</table>
**Hanging Drop Method:**

The hanging drop vapor diffusion method is one of the most popular techniques for crystallization condition screening and optimization. It is economical and is a reasonably fast procedure to carry out.

1. Using a disposable pipette, apply a thin layer of oil around the rim of each well (reservoir) in the Linbro plate where you intend to set up screening conditions (rows A-D, columns 1-5). Try not to get any oil inside of the wells.
2. Use your fingers to pick up the cover slips (hold them on the sides to prevent contamination from the oils on your skin) and lay out five of them on a clean surface.
3. Pipette 5uL of HIV-1 protease w/ inhibitor (solution “E”) onto the center of each cover slip. Using a FRESH pipette tip, add 5uL of the solution from the first well (A1) to the first drop on the first cover slip. Gently pipette up and down to homogenize the mixture.
4. Pick up the cover slip and invert, without losing the drop, over the first well. The oil will form a seal between the slip and the top of the well.
5. Repeat steps 3 and 4 for wells A2 to A5.
6. Repeat steps 1-5 for wells C1-C5.

**Sitting Drop Method- Micro-Bridges**

Sitting drop vapor diffusion is another popular method for crystallization condition screening and optimization. It allows you to use large volumes and is an even easier setup than the hanging drop.

1. Same as “Step 1” above in the hanging drop method.
2. Using your fingers, place a micro-bridge in wells B1-B5 and D1-D5.
3. Pipette 5uL of HIV-1 protease w/ inhibitor (“E”) into the depression at the center of each micro-bridge. Using a fresh tip, add 5uL of the solution from well B1 to the drop in the micro-bridge in B1. Gently pipette up and down to homogenize the mixture.
4. Pick up a clean cover slip and invert it over the well to create a seal between the slip and the top of the well.
5. Repeat steps 3 and 4 for wells B2-B5.
6. Repeat steps 1 to 5 for wells D1-D5.
1. What do you observe in the wells after they have been setup for 24 hours? Record your observations for each well below.

<table>
<thead>
<tr>
<th></th>
<th>0.4M NaCl</th>
<th>0.8M NaCl</th>
<th>1.0M NaCl</th>
<th>1.2M NaCl</th>
<th>1.6M NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanging Drop</td>
<td>“Well C1”</td>
<td>“Well C2”</td>
<td>“Well C3”</td>
<td>“Well C4”</td>
<td>“Well C5”</td>
</tr>
<tr>
<td>Sitting Drop</td>
<td>“Well D1”</td>
<td>“Well D2”</td>
<td>“Well D3”</td>
<td>“Well D4”</td>
<td>“Well D5”</td>
</tr>
</tbody>
</table>

2. How has the salt concentration affected the formation of crystals?
3. How has the PEG% affected the formation of the crystals?

4. Does it matter if the drops are hanging or sitting? Did one method work better than the other?

5. Do you think the ideal crystallization conditions would be the same for HIV-1 protease wild type versus mutant type?

6. Once again, your boss is asking about your results. Because he was pleased with the results from enzyme kinetics, he wants a poster of all the information to hang in the halls of the building showing off what has been done. A standard format that can be used on a poster is:
   - Name
   - Institution name
   - Title of work (remember, you are including information from bacterial transformation, enzyme kinetics, and crystallization).
   - Abstract- A summary of each section below. A good rule of thumb is to use one sentence from the background, procedures, results, and conclusion- although they can be restated.
   - Background information- Information about HIV-1 protease, why it is important to study.
   - Procedures- What exactly you did. Summarize the steps of bacterial transformation/cloning, enzyme kinetics, crystallization. DO NOT INCLUDE THE RESULTS IN THIS SECTION
   - Results- What are the results of your procedures? Include pictures, kinetic graphs, and figures with proper labels.
   - Discussion and conclusion- a short conclusion based on your research of what drug is best, and what the ideal crystallization conditions are.
   - Acknowledgement and references. List any references and acknowledge any help received.

All of this has to fit on a standard poster board (computer paper print-outs pasted to the board is recommended). Be concise and thorough. Keep in mind, it will be posted in the halls and everyone is going to see it!
**1.** What do you observe in the wells after they have been setup for 24 hours? Record your observations for each well below.

<table>
<thead>
<tr>
<th></th>
<th>0.4M NaCl</th>
<th>0.8M NaCl</th>
<th>1.0M NaCl</th>
<th>1.2M NaCl</th>
<th>1.6M NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanging Drop</td>
<td>“Well C1”</td>
<td>“Well C2”</td>
<td>“Well C3”</td>
<td>“Well C4”</td>
<td>“Well C5”</td>
</tr>
<tr>
<td>Sitting Drop</td>
<td>“Well D1”</td>
<td>“Well D2”</td>
<td>“Well D3”</td>
<td>“Well D4”</td>
<td>“Well D5”</td>
</tr>
</tbody>
</table>

2. How has the salt concentration affected the formation of crystals?
*There are different concentrations of salt that worked and concentrations that did not. I need to try this lab again to get an exact answer.*

3. How has the PEG% affected the formation of the crystals?
There are different amounts of PEG that worked and concentrations that did not. I need to try this lab again to get an exact answer.

4. Does it matter if the drops are hanging or sitting? Did one method work better than the other? 
   Not necessarily. Crystals formed in both, but this answer might change from student to student.

5. Do you think the ideal crystallization conditions would be the same for HIV-1 protease wild type versus mutant type? 
   Most likely they would not be the same. It may be possible, but unlikely. Different proteins require different environments in order to crystallize.