# Natural Products and Biosensors

*Preparing Extractions and Measuring Bioactivity*

## Pre-visit Preparation

Welcome to the University of Maryland Biotechnology Institute's (UMBI) SciTech Education Program. We hope that these materials are useful and will help prepare your students for a unique and exciting "hands-on" lab experience. We encourage you to review these pre-visit materials. The teacher background sheets are designed to increase your understanding of this topic. Students will have a richer experience with us if you go over the pre-visit materials with your class before your visit. For more information regarding SciTech, visit our website*: www.umbi.umd.edu/~scitech*
We encourage you to make copies of the pre-visit information for students and review them prior to your SciTech visit.

## Summary of Student Experience

After a brief introduction about natural products and their uses, the students will begin their experiment in the SciTech Lab. They will be provided with all of the equipment and supplies necessary for this lab, including a mortar and pestle, a centrifuge, a micropipet, natural products, bioluminescent bacteria and a lab worksheet. The students will then form their hypothesis and begin their experiment. The students will grind up a measured amount of a natural product, such as thyme, lavender or sunflower leaves, and make dilutions that will be tested on bioluminescent bacteria called *Photobacterium phosphoreum*. The students will test their extraction to determine if it has any antibacterial properties. Upon request, a Center of Marine Biotechnology (COMB) scientist will discuss his or her research, personal science career path and respond to student questions about possible careers in science.

## Tips for a Successful SciTech Experience

Natural products are used everyday for various reasons. Ask your students to think about what natural products they use in their everyday lives. For example, several different natural products that will breakdown protein can be found at the grocery store. Here are a few hints… Fresh fruit, eyecare, spices, laundry detergents.

Once you have found a variety of natural products that may break down protein, they can be tested using Knox Blocks gelatin. Prepare the Knox Blocks as per the package directions. Have the students prepare a simple lab experiment to test the effectiveness of each product in breaking down the protein in Knox Blocks. Record the results.

# Experimental Design Background

## Stating the Question

Every experiment begins with a question that the experiment will be designed to answer. Formulating this question can be a challenging part of setting up a research project. There are many questions that could be investigated during the exploration of the anti-bacterial activity of natural products. One such question could be "How can biosensors be used to test natural products for anti-bacterial activity?" Hopefully, many questions, including the one above, will be answered during the lab experience.

## Hypothesis

The clearest way to write a hypothesis is to use "if…then" statements. For example: "If a natural product is effective then the biosensor will be not be able to function properly." The most common hypothesis is the *null hypothesis* that simply states that the variable or experimental situation being tested will exhibit no significant difference from the controls. For example, one null hypothesis could state "There will be no observable difference between biosensors treated with natural products and biosensors treated with water."

## Controls

When designing an experiment, it is important to plan ahead so that the method you are testing is compared against a standard. If we will investigate how biosensors can be used to test the effectiveness of natural products then there need to be standards of comparison in the experiment. In our experiment we will use positive (alcohol) and negative (water) controls as a way of comparing the results from the student-prepared natural products.

# Natural Products Background

Living organisms produce an incredible variety of natural products. Nearly all of the Kingdoms have members that produce useful bioactive products. Examples of organisms that make useful natural products include bacteria, marine snails, orchids, algae, frogs and many others.

One ancient animal living in Chesapeake Bay is the common horseshoe crab, *Limulus polyphemus*. This animal has blood that contains an important enzyme, limulus amoebocyte lysate (LAL). LAL is used to detect bacterial contamination of medical supplies and media.

**Why Are Natural Products Important?**

If you have ever gotten sick and taken any sort of medicine to help you recover, you should probably thank a bacterium or a plant. Bacteria and plants are good sources of medicine because, since they cannot avoid predation by running away, they have evolved elaborate chemical defenses against their enemies. In plants, many of these defenses are trace phytochemicals that have biological activity useful in the treatment of human disease. There are over 300,000 known plant species, and more are still being identified. Only 5,000 of these plant species have been studied for medicinal usefulness. One of the reasons we hear frequent lament about the decimation of the tropical rain forests is that over 50% of all plant and animal species exist in tropical rain forests, including thousands of unidentified tropical plant species being lost forever. We will never know how many of these species could have produced useful natural products.

There is a similar problem closer to home. The U.S. spends millions of dollars on weed control without regard to potential medicinal use of products from those weeds. The rapid destruction of plants in the world increases the pressure to screen a large variety of plant species for bioactive properties before they become extinct. The Natural Products Division of the National Cancer Institute, at Fort Detrick in Frederick, MD, screens plant and animal species each year in an effort to identify potential pharmaceuticals. The screening process is intensive and may yield bioactive products from only 3 or 4 organisms from an initial group of a few thousand.

One of the most promising pain reducing drugs recently presented to the Food and Administration for approval comes from a venomous marine snail, *Conus magnus*, which lives in the Philippines. The reefs and marine environments of this island nation are considered some of the most threatened in the world. SNX-111 is the drug produced from an extraction of the snail's venom and it is beneficial because it is not addictive and does not have the narcotic effects of current pain killers such as

morphine. After approval by the FDA, this drug may be used to treat pain in cancer and AIDS patients. Because it is not a narcotic, it may have promising future uses as epidurals given to women during childbirth.

A company called Associates of Cape Cod manufactures and sells limulus amoebocyte lysate (LAL), which is produced by the horseshoe crab, *Limulus polyphemus*. LAL contains proteins that react to form a gel clot when exposed to bacterial endotoxins that are part of the cell wall of gram-negative bacteria. These toxins are called pyrogens because they cause a pyrogenic response (a fever) in humans. Endotoxins that enter the blood stream or spinal fluid in sufficient quantities can cause fever, shock, and even death. Because of LAL's unique properties, Associates of Cape Cod supplies this product to manufacturers of IV solutions, pharmaceuticals and other medical devices, so that they can test their products for harmful bacterial contamination. This test is rapid, sensitive, specific and inexpensive.

Another extraction from *Limulus polyphemus* blood currently being researched by the Associates of Cape Cod is Endotoxin Neutralizing Protein (ENP). This protein binds with the endotoxins and neutralizes their biological activity – so this product could be used to stop bacterial contamination as well as test for it.

The search for natural products has rapidly expanded to include plants, invertebrates and microbes from the marine world. Several promising marine natural products come from animals whose habitats are threatened and whose populations are in serious decline. Others have been identified from organisms that live at the deep-sea vents, and the promise for more natural products from marine organisms is optimistic. Today the ocean environment is threatened by many factors, including pollution, over-fishing and the rapid residential development of coastal ecosystems, so it is essential that we increase our awareness of these issues and accelerate the process of searching for natural products.

**Biosensors**

Like the "canary in the coal mine", modern scientists have turned to biosensors such as bioluminescent bacteria to act as sensitive sensors of environmental conditions. Natural products that have an anti-microbial effect may disrupt the bioluminescence produced by bacteria, indicating the presence of a bioactive compound. This is a way of screening for anti-bacterial properties of natural products. In the lab, you will use a bioluminescent bacterium *Photobacterium phosphoreum* as a biosensor to test the natural products that your students will prepare.

**Other Helpful Information**

The World Health Organization (WHO) reports that 85% of the world's population still uses herbs as their main form of medical treatment. Interest in medicinal plants has a long history. The Sumerians, who built the first city in southwest Asia 6,000 years ago, developed the oldest known medicinal handbook, a clay tablet which lists symptoms and prescriptions for medicinal plants and animal parts.

Students might be interested to know that women have always been considered healers. Throughout history, women had charge of matters concerned with food supply and medicines. They searched for wild roots and fruit to eat and medicinal plants for healing. Once termed "grandmother's secrets", these remedies are being validated by pharmacological research.

Ethnobotanist Memory Elvin-Lewis of Washington University speaks of her quest for medicinal plants as "finding a golden needle in the haystack." One of the ways to find this "golden needle", is through empirical selection – try it and see if it works. Root healers in the early 1900's used this method which fell into disrepute after several people died. A movement towards "safe and pure" medicinal chemicals ensued and the U.S. Food and Drug Administration (FDA) was formed.

Today, government oversight and regulation ensures quality and safety in the manufacturing of natural products or pharmaceuticals. Any natural product that is synthesized or purified in any way must pass government regulations.

# Glossary

**Bioactive** A substance that has a specific reaction on a biological system.

**Bioluminescence** The production of light by living organisms.

**Biosensor** A living organism that, because of its ability to detect and/or react to environmental changes, is used as a device to measure such changes.

**Centrifuge** An apparatus consisting of a compartment spun about a central axis to separate materials of different densities (liquids and solids).

**Dilution** A substance that has been reduced in concentration.

**Extraction** Something obtained from a substance by chemical or mechanical action.

**Homogenate** A mixture that is uniform in structure or composition throughout.

**Micropipet** A device used to measure minute amounts of a liquid.

**Pyrogen** A substance that produces fever in humans.

**Supernatant** The liquid portion of a sample that has been separated using a centrifuge.

## Web References


http://cancerweb.ncl.ac.uk/cancernet

http://www.mbl.edu/animals/Limulus/blood/bang.html

http://www.nigms.nih.gov/news/science_ed/medbydes.html

http://ibc.wustl.edu:70/0h/dbbs_book/Elvin-Lewis_M.html

# Maryland State Department of Education
# Core Learning Goals

The following Core Learning Goals link directly to the SciTech Natural Products and Biosensors Lab. Take a few moments to review the specific goals, expectations, and indicators below so that you may prepare your students appropriately. If you do not have a Core Learning Goals document or CD, talk to your department chair, contact your science supervisor, or visit the website http://www.mdk12.org/mspp/high_school/what_will/science/index.html.

## Core Learning Goal – Science

### *Goal 1 - Skills and Processes*

**Expectation 1.2** - The students will pose scientific questions and suggest investigative approaches to provide answers to questions.

*Indicator 1.2.1* -
- The student will identify meaningful, answerable scientific questions.

*Indicator 1.2.2* –
- The student will pose meaningful, answerable scientific questions.

*Indicator 1.2.3* -
- The student will formulate a working hypothesis.

*Indicator 1.2.4* -
- The student will test a working hypothesis.

*Indicator 1.2.5* -
- The student will select appropriate instruments and materials to conduct an investigation.

**Expectation 1.3** - The student will carry out scientific investigations effectively and employ the instruments, systems of measurement, and materials of science appropriately.

*Indicator 1.3.4* -
- The student will learn the use of new instruments and equipment by following instructions in a manual or from oral direction.

**Expectation 1.4** - The student will demonstrate that data analysis is a vital aspect of the processes of scientific inquiry and communication.

*Indicator 1.4.9* -
- The student will use analyzed data to confirm, modify, or reject an hypothesis.

**Expectation 1.5 -** The student will use appropriate methods for communicating in writing and orally the processes and results of scientific investigation.

*Indicator 1.5.1 -*

- The student will demonstrate the ability to summarize data (measurements/observations).

*Indicator 1.5.2 -*

- The student will explain scientific concepts and processes through drawing, writing, and/or oral communication.

**Expectation 1.7 -** The student will show that connections exist both within the various fields of science and among science and other disciplines including mathematics, social studies, language arts, fine arts, and technology.

*Indicator 1.7.5 -*

- The student will investigate career possibilities in the various areas of science.

***Goal 3 - Concepts of Biology***

**Expectation 3.2 -** The student will demonstrate an understanding that all organisms are composed of cells which can function independently or as part of multi-cellular organisms.

*Indicator 3.2.2 -*

- The student will conclude that cells exist within a narrow range of environmental conditions and changes to that environment, either naturally occurring or induced, may cause changes in the metabolic activity of the cell or organism.

**Expectation 3.5 -** The student will investigate the interdependence of diverse living organisms and their interactions with the components of the biosphere.

*Indicator 3.5.3 -*

- The student will investigate how natural and man-made changes in environmental conditions will affect individual organisms and the dynamics of populations.