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**April/May 2015 Teacher's Guide for**

***Venoms: From Lethal to Life-Saving***

**Table of Contents**

[About the Guide 2](#_Toc415151668)

[Student Questions 3](#_Toc415151669)

[Answers to Student Questions 4](#_Toc415151670)

[Anticipation Guide 6](#_Toc415151671)

[Reading Strategies 7](#_Toc415151672)

[Background Information 9](#_Toc415151673)

[Connections to Chemistry Concepts 17](#_Toc415151674)

[Possible Student Misconceptions 18](#_Toc415151675)

[Anticipating Student Questions 18](#_Toc415151676)

[In-Class Activities 19](#_Toc415151677)

[Out-of-class Activities and Projects 20](#_Toc415151678)

[References 20](#_Toc415151679)

[Web Sites for Additional Information 21](#_Toc415151680)

[More Web Sites on Teacher Information and Lesson Plans 23](#_Toc415151681)

# About the Guide

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Articles from past issues of *ChemMatters* can be accessed from a DVD that is available from the American Chemical Society for $42. The DVD contains the entire 30-year publication of *ChemMatters* issues, from February 1983 to April 2013.

The *ChemMatters* DVD also includes Article, Title and Keyword Indexes that covers all issues from February 1983 to April 2013.

The *ChemMatters* DVD can be purchased by calling 1-800-227-5558.

Purchase information can be found online at [www.acs.org/chemmatters](http://chemistry.org/chemmatters/cd3.html).

# Student Questions

**(taken from article)**

* 1. How is snake antivenom produced?
  2. What are the new uses of animal venom in medicine?
  3. What is venomics?
  4. Venom contains proteins which are composed of amino acids. Describe the chemical composition of amino acids.
  5. What are some of the different toxins found in venom, and what do they do to a victim of a snake-bite?
  6. What is the difference between the way morphine works to dull pain and how a derivative of mamba venom blocks pain?
  7. What animal is the source of the pain medication Ziconotide?
  8. How are the two anticoagulant drugs derived from viper venom, ancrod (Arwin) and tirofiban (Aggrastat), used in medicine?
  9. What is the source of chlorotoxin?
  10. How is chlorotoxin used in medicine?
  11. What techniques are used to synthesize needed compounds from spider or scorpion venom?

# Answers to Student Questions

**(from article)**

* + 1. **How is snake antivenom made?**

*Dilute doses of a particular venom are injected into animals. Antibodies to the venom are then produced in the animals’ blood. The antibodies are isolated and are then used as the antivenom injected into the victim of a snake bite.*

* + 1. **What are the new uses of animal venom in medicine?**

*Different venoms or their altered components have been found to be effective in treating such medical conditions as high blood pressure, diabetes and even cancer.*

* + 1. **What is venomics?**

*Venomics is the practice of synthesizing in the lab venom toxins or some of their components either for treating snake bites or other medical conditions mentioned previously.*

* + 1. **Venom contains proteins which are composed of amino acids. Describe the chemical composition of amino acids.**

*Amino acids contain carbon, hydrogen, oxygen and nitrogen. These atoms are arranged into groups called carboxyl (COOH) and amine (NH2) groups that are involved in bonding between the various amino acids in a protein molecule.*

* + 1. **What are some of the different toxins found in venom, and what do they do to a victim of a snake-bite?**
       1. *One toxin, called hemotoxin, destroys red blood cells, which can disrupt blood clotting and damage the heart.*
       2. *Another, called cardiotoxin, can cause the heart to speed up or to stop dead.*
       3. *Neurotoxins interrupt signals from nerves to muscles and the brain.*
       4. *Myotoxins paralyze muscles, preventing a prey’s escape or breathing.*
    2. **What is the difference between a) the way morphine works to dull pain and b) how a derivative of mamba venom works to block pain?**
       1. *Morphine molecules bind to receptors, proteins, on the surface of spinal neurons, setting off chemical reactions inside the neuron that cause the neurons to fire less, preventing the transfer of pain signals.*
       2. *Two proteins (named mambalgins) that are derived from mamba venom bind to different proteins on a spinal neuron’s surface membrane. These proteins are part of what are called ion channels. These channels are small tubes through which ions travel, helping to relay pain messages. The mambalgins block these ion channels, hence preventing a pain signal from being created in the neuron.*
    3. **What animal is the source of the pain medication Ziconotide?**

*Ziconotide is made from the venom of the cone snail.*

* + 1. **How are the two anticoagulant drugs derived from viper venom, ancrod (Arwin) and tirofiban (Aggrastat), used in medicine?**

*Arwin is used to prevent clots in the brains of patients who have suffered from a stroke in which the clots have blocked some of the blood vessels to the brain, reducing oxygen flow.  
Aggrastat is used to stop blood from clotting during a heart attack or heart surgery.*

* + 1. **What is the source of chlorotoxin?**

*Chlorotoxin is found in the venom of the death stalker scorpion.*

* + 1. **How is chlorotoxin used in medicine?**

*Chlorotoxin is used to label cancer cells which can then be seen due to its ability to glow when exposed to an infrared camera, which in turn allows a surgeon to remove any glowing cancer cells.*

* + 1. **What techniques are used to synthesize needed compounds from spider or scorpion venom?**

*Researchers can either synthesize a particular protein in venom using its component amino acids (building blocks), or a bacterium can be genetically engineered to produce large quantities of a particular venom protein.*

# Anticipation Guide

Anticipation guides help engage students by activating prior knowledge and stimulating student interest before reading. If class time permits, discuss students’ responses to each statement before reading each article. As they read, students should look for evidence supporting or refuting their initial responses.

**Directions:**  *Before reading*, in the first column, write “A” or “D,” indicating your agreement or disagreement with each statement. As you read, compare your opinions with information from the article. In the space under each statement, cite information from the article that supports or refutes your original ideas.

|  |  |  |
| --- | --- | --- |
| **Me** | **Text** | **Statement** |
|  |  | 1. Antivenom to treat snake bites comes from snake bite victims. |
|  |  | 1. Toxins found in venoms are being synthesized to treat several conditions and diseases. |
|  |  | 1. Venoms contain carbohydrates. |
|  |  | 1. Proteins are chains of amino acids. |
|  |  | 1. Each kind of venom contains only one type of toxin. |
|  |  | 1. Proteins from black mamba venom can stop pain in mice. |
|  |  | 1. The first drug based on snake venom was developed in the 1990s. |
|  |  | 1. Venoms may come from snakes, snails, spiders, and scorpions |
|  |  | 1. Drugs may be genetically engineered using bacteria. |
|  |  | 1. Drugs developed from venoms are often destroyed in the stomach. |

# Reading Strategies

These graphic organizers are provided to help students locate and analyze information from the articles. Student understanding will be enhanced when they explore and evaluate the information themselves, with input from the teacher if students are struggling. Encourage students to use their own words and avoid copying entire sentences from the articles. The use of bullets helps them do this. If you use these reading and writing strategies to evaluate student performance, you may want to develop a grading rubric such as the one below.

|  |  |  |
| --- | --- | --- |
| **Score** | **Description** | **Evidence** |
| 4 | Excellent | Complete; details provided; demonstrates deep understanding. |
| 3 | Good | Complete; few details provided; demonstrates some understanding. |
| 2 | Fair | Incomplete; few details provided; some misconceptions evident. |
| 1 | Poor | Very incomplete; no details provided; many misconceptions evident. |
| 0 | Not acceptable | So incomplete that no judgment can be made about student understanding |

***Teaching Strategies:***

1. Links to **Common Core Standards for Reading**:
   1. ELA-Literacy.RST.9-10.5: Analyze the structure of the relationships among concepts in a text, including relationships among key terms (e.g., force, friction, reaction force, energy).
   2. ELA-Literacy.RST.11-12.4: Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 11-12 texts and topics.
2. Links to **Common Core Standards for Writing**:
   1. ELA-Literacy.WHST.9-10.2F: Provide a concluding statement or section that follows from and supports the information or explanation presented (e.g., articulating implications or the significance of the topic).
   2. ELA-Literacy.WHST.11-12.1E: Provide a concluding statement or section that follows from or supports the argument presented.
3. **Vocabulary** and **concepts** that are reinforced in this issue: Chirality; Enantiomer; Amino acid; Protein; Enzyme; and Organic molecular structures.
4. To help students engage with the text, ask students which article **engaged** them most and why, or what **questions** they still have about the articles. The Background Information in the *ChemMatters* Teachers Guide has suggestions for further research and activities.

***Directions*:** As you read the article, complete the graphic organizer below to describe how venoms may be used to treat various conditions and diseases.

|  |  |  |  |
| --- | --- | --- | --- |
| **Use for compound found in venom** | **Source of possible venoms used** | **How it works** | **Advantage** |
| **Painkiller** |  |  |  |
| **Prevention of blood clots (anticoagulants)** |  |  |  |
| **“Tumor paint”** |  |  |  |
| **Current and future work to develop drugs** |  |  | |

# Background Information

**(teacher information)**

**More on neurophysiology of pain**

The great interest in developing venom molecules for alleviating pain requires an understanding of how pain is manifested in humans. The essence of “feeling” pain is what happens in the nerves of the spinal cord, specifically the dorsal root ganglia from which nerve impulses generated by nerve endings in the skin and other body structures are sent to the spinal cord and the brain. Genetic mutations or damage to nerves can change the behavior of key molecules along the route from the original pain receptors. Embedded in the membranes of nerve endings that detect pain stimuli are protein molecules that make up the structure of what are called ion channels. These channels control the flow of ions such as potassium, sodium, and chloride which are responsible for generating a nerve impulse due to changes in their concentration inside and outside a nerve cell.

Nearly 50 years ago researchers showed that electrical activity in neurons is produced by subtle changes in the neuron's potassium concentration…. Since then, it's been well established that the flow of potassium ions is central to many different cellular processes…. Potassium currents in the brain, for example, underlie perception and movement, and the heart's contraction relies upon the steady ebb-and-flow of potassium…. To maintain the correct concentration of potassium, cells are equipped with pore-like proteins that poke through the cell membrane. These proteins, called ion channels or potassium channels, create sieves through which potassium ions flow from inside to outside the cell.

(<http://www.hhmi.org/news/visualizing-potassium-channel>)

In humans, 78 unique potassium channel genes underlie an impressive level of functional diversity. The different potassium channels have evolved the ability to open—by a process called gating—in response to different forces in their environment. Certain potassium channels are gated by the binding of calcium ions, specific membrane lipids, or G protein subunits. Others respond to mechanical perturbations of the membrane or changes in voltage across the membrane. Thus, the potassium channels control a cell's electrical state through many different avenues.

(<http://www.hhmi.org/research/structure-and-mechanisms-ion-channels>)

These changes in concentrations change the polarity of the nerve cell which is associated with generating a nerve impulse. For example, an ion channel designated as TRPV1 detects heat. When it opens, positive ions such as sodium rush into the nerve cell from the surrounding interstitial fluid. This influx of sodium ions changes the membrane voltage which in turn opens specific sodium channels. This influx further changes the membrane voltage which generates a pain signal (nerve signal) from the pain sensors to the spinal cord, then on to the brain for interpretation. So, if one can find molecules that can block these changes that begin with the ion channels opening up, then one can prevent the ultimate generation of a pain signal to the brain.

And this is where the extracts of certain molecules found in venom, most often protein-based, fit in. Certain venoms contain molecules that affect these ion channels. Some actually keep the ion channels open which means that nerve impulses are continuously generated, producing immobility through a type of paralysis. The muscles never relax, simply contracting repeatedly (a catatonic state). Different molecules from venom do the reverse—they block the inflow of ions which is necessary to ultimately effect a nerve impulse, therefore preventing any pain signal from being generated. These venom extracts do not carry with them the several undesirable side effects that are associated with morphine and other pain-killing narcotics.

The difference between the venom-based pain killers and the narcotic derivatives is their locus of operation. The narcotics act on the surface of the spinal cord membrane, therefore affecting many aspects of the spinal cord’s nervous activities, which includes many different receptors in the brain. But targeting only the pain receptors via the ion channels affects only one area of the brain. And there does not seem to be the other side effects of narcotics, including addiction as well as the fact that one’s tolerance for the drug increases—which means more and more narcotic is needed to produce the same effect in terms of pain reduction. (It is said that more Americans die from an overdose of prescription pain killers than from overdoses of cocaine and heroin combined.)

The sodium ion channels are of interest to researchers as a target for controlling pain. It is known that novocaine and lignocaine (lidocaine) block a variety of sodium channels rather than just one or two specific ones. That results in blocking not only pain sensations but all other sensations where applied. It is preferable to block specific channels that control pain, rather than all the sodium channels. There are nine different voltage-controlling channels found in humans (and other mammals) that respond to slightly different voltages generated within a nerve cell. It is important that a particular chemical developed to control a sodium channel be very specific— affecting all the sodium channels would mean shutting down a whole host of nerve-controlled activities as diverse as heartbeat, breathing, and movement. There are several sensory-specific sodium ion channels that are of interest. They are designated Nav1.7, 1.8, and 1.9. (The Na designation refers to sodium.) Each of these channel types has a different voltage-sensing area in the channel, which means that any pain-relieving medicines would be specific to one of the channels.

One research group at Duke University has found a particular antibody of the immune system that affects the Nav1.7 channel, and not only blocks inflammatory pain (e.g., arthritic pain) and neuropathic pain (pain from nerve damage caused by injury, disease or another insult), but also alleviates itching! Ion channels have a protein component to which a specific antibody can react (bind).

The “detection” or interpretation of pain sensations involves specific locations in the brain that have been more accurately identified in recent years. But, it is also true that because of the involvement of the brain, there are different types of pain that are given “reality” by the role of the brain’s interpretation.

Even as researchers make strides in understanding and treating pain, new discoveries raise many more questions. These recent findings reveal the deep connections between pain and many essential physical and mental processes.

* Patients with chronic back pain tend to be impaired at emotional learning but have increased sensitivity to taste.
* Chronic pain shrinks the brain, up to 11 percent in some cases.
* Those with chronic pain can learn to control their perception of pain by imagining pleasant scenarios or believing a particular stimulus to be harmless.
* Memory of a pain can cause that pain to persist for life, even after the initial injury has healed.
* Chronic pain sufferers can learn to associate a place with their pain. Returning to this space can reinforce the negative association.

(<http://www.scientificamerican.com/article/neuroscientists-identify-brain-signature-pain/>)

**More on** **venom**

Speaking of channel blocking, venoms turn out to have certain molecular components that have just the right shape (specificity) and chemical activity to latch onto one part of a voltage-sensing area of a sodium channel and control (block) its activity which is necessary to generate a nerve impulse within a nerve cell. Several sources of venom-derived channel blockers include two pain-killing molecules from the venom of the black mamba (mentioned in the *ChemMatters* article); however, these “mambalgins”, as they are called, do not block sodium channels but rather those that are known as acid-sensing ion channels (ASIC). In this particular case, protons (H+) do the blocking, such that no nerve impulse is generated.

When we’re injured, our damaged cells release an “inflammatory soup” of chemicals that triggers feelings of pain. Among the first of these harbingers are simple protons – positively charged particles that make the local tissues more acidic. The ASICs detect and respond to protons by opening up, allowing positive ions to flood inside, and causing the neurons to fire. They’re warning systems that tell our bodies that something is wrong.

Diochot and Baron found two peptides (short proteins) from black mamba venom that block ASICs—mambalgin-1 and mambalgin-2. They act as padlocks that latch onto the closed proteins and stop them from opening, even when surrounded by protons. And they have characteristics that are almost too good to be true.

They work quickly and effectively against every type of ASIC found in our nervous system. As painkillers, they’re as potent as morphine. They’ll numb the sharp pain of a burn, as well as the dull throb of an inflamed limb. They’re incredibly specific: they don’t stop neurons from firing more generally, and they don’t block any of the other gate-keeping proteins found in these cells. And unlike other similarly shaped proteins, they don’t have any toxic effects, such as paralysis, convulsions or breathing difficulties.

[The list of side effects that Diochot and Baron checked for, and saw no sign of, includes “death”; good to know.]

(<http://blogs.discovermagazine.com/notrocketscience/2012/10/03/painkilling-chemicals-with-no-side-effects-found-in-black-mamba-venom/#.VOOUVyyLGo1>)

An interesting side issue with regard to developing a pain-killing drug is that the drug must be able to survive a person’s degrading digestive juices when ingested in pill form. The importance of molecular design in resisting these destructive effects of the digestive system is illustrated in the case of the development of a pain-killing drug called Ziconotide. This drug, which is based on a molecule isolated from the toxin of the cone snail, will not survive intact in a patient’s digestive system. Therefore, it has to be administered as an injection into the spinal cord. David Craik, an Australian biochemist, is re-engineering this drug so that it cannot be broken down in the digestive system. He has changed the drug from a straight chain, whose ends can easily be “snipped off” chemically by enzymes, to a ring structure which is more stable (i.e., lacking the unprotected ends). He has given oral doses to rats and has found it to be 100 times more potent than a drug called gabapentin, a common treatment for nerve pain.

**More on** **ion channels**

In connection with research to isolate substances from venom, it is found that one venom from the cone snail that normally paralyzes the muscle of fish and amphibians does not do so in mammalian tissue, which includes that of humans. The peptide in this venom blocks the flow of calcium from outside of a nerve cell into the interior of the cell. Normally, this flow into the cell causes the release of a neurotransmitter called acetylcholine, which flows out of the nerve cell into the region between the nerve cell and a muscle cell, causing a nerve impulse that stimulates the muscle cell. (A one-minute video that clearly illustrates the basics of a nerve impulse and the changes at a synapse to continue a nerve impulse into an adjacent nerve cell or muscle cell can be seen here: <http://www.hhmi.org/biointeractive/molecular-mechanism-synaptic-function>.) The stimulation produces a contraction of that muscle cell, which equals movement. Blocking the calcium channel into the nerve cell prevents the release of the acetylcholine, and no stimulation of a muscle cell takes place (which results in paralysis of movement). But the important thing is that this same venom peptide does NOT block muscle cells in mammalians (as it does in fish and amphibians), but it does block calcium channels in nerve cells that belong to what are called pain fibers. Again, blocking the flow of calcium from outside the nerve cell into its interior means that no acetylcholine is released to stimulate an adjacent nerve cell that normally transmits a pain signal to the central nervous system. This set of short videos with visual explanations clearly illustrate the mechanism by which this venom peptide operates at the cellular level: <http://www.hhmi.org/biointeractive/prialt-blocks-motor-synapse-fish> and <http://www.hhmi.org/biointeractive/prialt-blocks-pain-signaling-mice>.

In addition, a 30-second video that shows the structure of an ion channel and how it changes shape (related to blocking or transmitting an ion) in the presence of specific molecules, in this case capsaicin and a spider toxin, can be found at the site listed directly below. An explanation of the role of this structure and pain sensing is as follows:

That rapid-fire signal (from pain-sensing nerve receptors) begins in a heat-sensing molecule called a TRPV1 channel. This specialized protein is abundant on the surface of sensory nerve cells in our fingers and elsewhere and is a shape-shifter that can take an open or closed configuration. Heat opens a central pore in our fingers and elsewhere and is a shape-shifter that can take an open or closed configuration. Heat opens a central pore in the molecule, so do certain spider toxins and capsaicin—the substance that gives chili peppers their burn. Once the pore is open, charged ions of sodium and calcium flow into the nerve cell, triggering the pain signal.

(<http://www.scientificamerican.com/article/pain-research-reveals-the-structure-of-a-molecular-gateway-to-pain-animation/>)

A variety of inorganic and organic molecules can modulate ion channel activity and conductance. Some commonly used ion channel blockers found in different venoms or toxins include:

* [*Tetrodotoxin*](http://en.wikipedia.org/wiki/Tetrodotoxin) *(TTX), used by* [*puffer fish*](http://en.wikipedia.org/wiki/Puffer_fish) *and some types of* [*newts*](http://en.wikipedia.org/wiki/Newts) *for defense. It blocks sodium channels.*
* [*Saxitoxin*](http://en.wikipedia.org/wiki/Saxitoxin) *is produced by a* [*dinoflagellate*](http://en.wikipedia.org/wiki/Dinoflagellate) *also known as "*[*red tide*](http://en.wikipedia.org/wiki/Red_tide)*". It blocks voltage-dependent sodium channels.*
* [*Conotoxin*](http://en.wikipedia.org/wiki/Conotoxin) *is used by* [*cone snails*](http://en.wikipedia.org/wiki/Cone_snails) *to hunt prey. There are five different conotoxins that can block either sodium, potassium, or calcium channels. In addition one particular conotoxin can interfere with acetylcholine receptors in nerve cells.*
* [*Lidocaine*](http://en.wikipedia.org/wiki/Lidocaine) *and* [*Novocaine*](http://en.wikipedia.org/wiki/Novocaine) *belong to a class of* [*local anesthetics*](http://en.wikipedia.org/wiki/Local_anesthetics) *which block sodium ion channels.*
* [*Dendrotoxin*](http://en.wikipedia.org/wiki/Dendrotoxin) *is produced by* [*mamba*](http://en.wikipedia.org/wiki/Mamba) [*snakes*](http://en.wikipedia.org/wiki/Snakes)*, and blocks potassium channels.*
* [*Iberiotoxin*](http://en.wikipedia.org/wiki/Iberiotoxin) *is produced by the* [*Buthus*](http://en.wikipedia.org/wiki/Buthus) *tamulus (Eastern Indian scorpion) and blocks potassium channels.*
* [*Heteropodatoxin*](http://en.wikipedia.org/wiki/Heteropodatoxin) *is produced by* [*Heteropoda venatoria*](http://en.wikipedia.org/wiki/Heteropoda_venatoria) *(brown huntsman spider or laya) and blocks potassium channels.*

(<http://en.wikipedia.org/wiki/Ion_channel>)

**More on** **using venom as target-specific drugs (cancer treatment)**

What is it in venom that makes it a potentially useful cancer-treating substance? Venom from certain kinds of snakes, scorpions, and bees contain a number of compounds that, when delivered to cancer-stricken tissues, can destroy cancer cells in a number of ways. One way is by changing the permeability of the cells membrane, causing uncontrolled flow of certain ions in or out of the cell’s cyctoplasm. This creates a chemical imbalance that disrupts vital functions of various organelles within the cell. For example, an inflow of calcium ions (Ca2+) into the cell from the surrounding tissue fluids into the cytoplasm disrupts the functioning of the mitochondria, the energy-producing organelles. If it loses its energy production, the cell dies. That is the way cyanide poisoning works. Another effect is to cause the release of cytochrome *c* from the mitochondria. The release of the cytochrome, in turn, helps to activate caspases, a family of killer proteases. Proteases destroy proteins by disrupting peptide bonds. One example of an important class of proteins is enzymes, organic catalysts vital to cell physiology.

Changing cell membrane porosity also enhances the activity of a chemical called phospholipase A2. This chemical is found in snake and bee sting venom and can cause cell death through a number of chemical mechanisms. But one important way in which the phospholipase can destroy cancer cells is through its action of preventing the development of vascular tissue which is needed to nourish cancer cell growth. It also appears to be the case that the phospholipase can target certain proteins in cancer cells, making the chemical a directed substance in terms of reaching its intended destination, the cancer cells.

Additional proteins, peptides, and enzymes found in venom also can affect cancer cells by preventing them from migrating to other areas and attaching to normal cells for proliferation (metastasis). One particular molecule is called *contortrostatin*. Normally, snake venom causes excessive bleeding in victims by preventing coagulation of the blood. Coagulation occurs when blood cells clump together through the formation of a clotting material called fibrin. Using this same chemical mechanism from snake venom can prevent the cancer cells from attaching to normal tissue cells. The same chemical also blocks signals from the cancer cells to stimulate the development of vascular tissue needed to supply nutrients for growing cancer cells. Researchers have been able to produce large quantities of *contortrostatin* by genetically engineering certain bacteria to synthesize this specific protein, a common pharmacological technique. The process comes under the category of large vat fermentation.

It has also been found that certain venom components can cause biochemical reactions in cells that produce hydrogen peroxide, a cell killer.

**More on targeting cancer cells for drug delivery**

For any particular venom-based anticancer chemical that has been isolated, there has to be a method for delivering it to specific cancerous cells if the chemical is to be effective. As with other cancer drugs used in chemotherapy, it is preferable to deliver cancer drugs to specific locations rather than using a systemic approach as many of the drugs are toxic to healthy cells as well as cancerous cells. In some instances, the effects of venom-based drugs are specific only to cancer cells. But in addition, being able to target cancer cells means that concentrations of the drug can be kept higher than when they are injected in a systemic way. How can we make the drug latch onto cancer cells and not healthy cells?

Three methods for making drugs target-specific include using antigen-antibody matching (cancer cell-drug proteins), using RNA-based molecules on the drug to match the specific DNA on the surface of cancer cells, and using nanoparticles that can be designed to match the cell surface of cancer cells. In one case of using nanoparticles, there are actually two different nanoparticles—one type identifies and attaches to the cancer cell, while the second nanoparticle carrying the drug locates the first attached nanoparticle by detecting signals emitted by the attached nanoparticle. The encapsulated venom protein bypasses normal cells but its structure targets cancer cell structures. In each type of delivery system, the targeting carrier molecule contains the anti-cancer drug, be it a venom-derived series of molecules or some other chemical compound known to inhibit or destroy cancer cells.

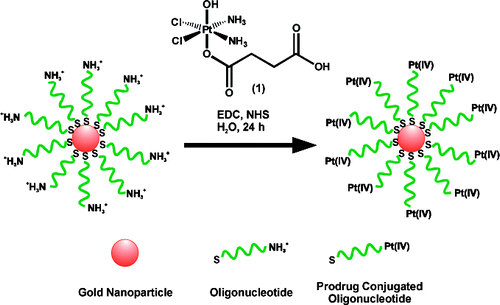
Some fine tuning for increasing the effectiveness of a cancer drug includes using a photosensitizing agent to enhance the ability of drug-carrying nanoparticles to enter tumors. First let the photosensitizing agent accumulate in the tumor, then illuminate the tumor with infrared light. The photosensitizing agent causes the blood vessels in the tumor to be more porous, therefore allowing more drug-carrying nanoparticles to enter the tumor. Another application of using nanoparticles is found in a skin cancer treatment in which RNA molecules are attached to gold nanoparticles. The nanoparticles are in an ointment that is applied to the skin. The nanoparticles penetrate the skin and the RNA attaches to a cancer-related gene, stopping the gene from generating proteins that are used in the growth of skin cancer tumors.

Another interesting bit of chemistry involved in chemotherapy using gold nanoparticles is described below.

Reporting its work in the *Journal of the American Chemical Society*, a team of investigators led by Stephen Lippard, of the Massachusetts Institute of Technology, and Chad Mirkin, of Northwestern University and principal investigator of the Northwestern Center for Cancer Nanotechnology Excellence, describes its development and characterization of gold nanoparticles as a delivery vehicle for a non-toxic form of platinum known as platinum(IV). The gold nanoparticles are coated with short pieces of nucleic acid to which the investigators chemically attach platinum(IV). This construct can circulate safely through the blood stream and readily enter tumor cells, which take up the nanoparticles and their payload by engulfing them in tiny pockets of cell membrane called endosomes. The acidic environment inside the endosome is such that platinum(IV) undergoes a chemical reaction that converts it into platinum(II), the highly toxic form of this element.

(<http://www.understandingnano.com/cancer-therapy-platinum-gold-nanoparticles.html>)

A diagram of this nano-delivery system is shown below.



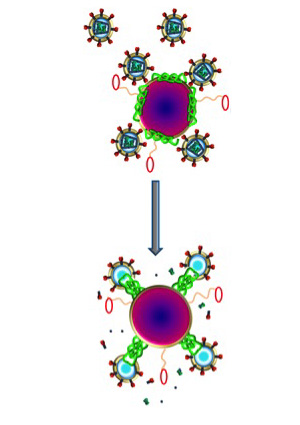
([*http://pubs.acs.org/doi/abs/10.1021/ja9071282*](http://pubs.acs.org/doi/abs/10.1021/ja9071282))

A set of goals for directing research efforts into developing cancer drug delivery systems includes the following:

Scientists are working to increase the availability of drug for tumor uptake by 1) delaying the release preparations for long-lasting actions; 2) using liposome-entrapped drugs for prolonged effect or reduced toxicity; 3) administrating inert, non-toxic prodrugs for specific activation at the tumor site; 4) delivering the antibody-mediated drugs; or 5) conjugating site-specific carriers to direct the drug to the tumor target.

(<http://www.ncbi.nlm.nih.gov/pubmed/2689812>)

**More sites on** **using nanotechnology to deliver a venom-derived toxin**



Bee venom that is injected when a bee stings contains a potent toxin called melittin. Researchers have found that this melittin can destroy HIV viruses. The article describes the methods by which the toxin can be delivered specifically to the virus and not harm normal cells. Nanotechnology provides a delivery mechanism for the melittin that latches onto the virus’s surface, allowing the melittin to destroy the virus’s double membrane. Many other viruses with the same double membrane (e.g., hepatitis B and C), may prove vulnerable to this same nanoparticle delivery system. Refer to <http://news.wustl.edu/news/Pages/25061.aspx>.

A diagram of how nanoparticles can be used to deliver the melittin to target cells is shown at right. What is of interest is the use of “bumper” molecules which prevent the nanoparticles from harming normal body cells.

*(Nanoparticles (****purple****) carrying melittin (****green****) fuse with HIV (small circles with spiked outer ring), destroying the virus’s protective envelope. Molecular bumpers (small* ***red*** *ovals) prevent the nanoparticles from harming the body’s normal cells, which are much larger in size.)*

([*http://news.wustl.edu/news/Pages/25061.aspx*](http://news.wustl.edu/news/Pages/25061.aspx))

**More on producing antivenom**

Producing antivenom to counter the effects of venom in both humans and other mammals (grazing animals can be bitten by venomous snakes with the same fatal results as in humans) is based on the same general process used for developing antibody-based vaccines. The scientist introduces small quantities of a particular antigen (a bacterium or virus) into a host animal which then responds through its immune system to produce a specific set of antibodies. These antibodies are then collected from the blood of the incubating animal and refined for inclusion in a vaccine medium. The following chart lists the various animals used for collecting antibodies and a specific venom. (Not all snake venoms have had anti-venom developed for treatment of a particular snake bite.

|  |  |  |
| --- | --- | --- |
| **Black Snake** | **Equine** | **Fab2 fragment** |
| **Box Jellyfish** | **Ovine** | **Whole Ig** |
| **Brown Snake** | **Equine** | **Fab2 fragment** |
| **Death Adder** | **Equine** | **Fab2 fragment** |
| **Funnel Web Spider** | **Leporine** | **Whole Ig** |
| **Polyvalent (land snake)** | **Equine** | **Fab2 fragment** |
| **Redback Spider** | **Equine** | **Fab2 fragment** |
| **Sea Snake** | **Equine** | **Fab2 fragment** |
| **Stonefish** | **Equine** | **Fab2 fragment** |
| **Taipan** | **Equine** | **Fab2 fragment** |
| **Tiger Snake** | **Equine** | **Fab2 fragment** |

(*source unknown*)

Australia was one of the first countries in the world to experiment with snake antivenoms through the pioneering work of Frank Tidswell in 1898. He experimented with inoculating a horse, using venom from a tiger snake. Through progressive increases in the amount of snake venom injected into a horse, he was able to determine the maximum amount of toxin that would produce enough antibodies without killing the horse. The blood serum containing the antibodies was then injected incrementally to determine the largest amount of serum needed to neutralize injected snake venom in an animal. He also found that the antivenom would work hours after an animal was exposed to the venom. (ref. <http://en.wikipedia.org/wiki/Frank_Tidswell>)

# Connections to Chemistry Concepts

**(for correlation to course curriculum)**

1. **Amino acids**—These building blocks for protein molecules can be identified and used to synthesize several of the important molecules found in venoms for treating pain, cancer, and heart conditions.
2. **Protein**—This particular class of molecule containing a variety of amino acids is often associated with structural molecules in living organisms, as well as with several functional molecules in venom that produce a variety of effects in animals when the venom becomes part of an animal’s physiology.
3. **Organic**—This large category of carbon-containing molecules includes things like venom, enzymes, antivenom (antibodies), and pain killer molecules derived from venom.
4. **Enzymes**—This important category of organic proteins that act as catalysts in biochemical reactions are sometimes the target of venom toxins which can inactivate or accelerate the enzyme action, to the detriment of certain cellular activities that depend on the enzyme.

# Possible Student Misconceptions

**(to aid teacher in addressing misconceptions)**

1. **“Snake charmers entice poisonous viper snakes, such as the cobra, out of their basket enclosures with music from a flute.”** *The snake charmer uses a flute and plays music, but snakes cannot hear sounds. What attracts the snake to come out of an enclosure is the movement of the charmer’s flute which the snake sees as some kind of a threat. Snake charmers capture snakes out in their normal habitat, sometimes defanging the snake or sewing the snake’s mouth nearly shut to prevent the handler from being fatally bitten. Fangs normally regrow quickly, and a snake with its mouth sewn shut will starve to death.*
2. **“Any snake venom can be used as an antivenom for any other snake bite.”** *Specific snakes have specific venoms that produce specific antibodies when injected into test animals. The specific antibody results in an antivenom that is specific to that snake. Using another snake antivenom for that snake’s bite will be ineffectual.*
3. **“If you’re bitten by a venomous snake, quickly suck the venom out of the bite and you’ll be OK.”** *Unfortunately, this only works in the movies. This method was suggested way back when, with the hypothesis being that you could suck out a lot of the venom before it enters the bloodstream. But the venom usually enters the bloodstream so quickly that it’s already been absorbed by the time you try to suck it out. Long and short of it: if you’ve been bitten, seek medical attention immediately. Here’s a reference:* [*http://adventure.howstuffworks.com/snake-bite.htm*](http://adventure.howstuffworks.com/snake-bite.htm)*.*

*[Editor RT note: A precautionary method for walking in the bush in East Africa is to use a walking stick to pound the ground as you walk in grassy areas to alert (scare away) mamba snakes. They are quick movers.]*

# Anticipating Student Questions

**(answers to questions students might ask in class)**

1. **“Does the use of an ‘epi-pen’ involve an antibody vaccine against a bee sting?”** *No, the epi-pen contains epinephrine which actually counters the reaction of a person’s immune system’s response to the antigen of a bee sting, the reaction being primarily the shutdown of the respiratory system. The smooth muscle around the alveoli in the lungs constrict, restricting a victim’s ability to exhale. A snake antivenom injection provides specific antibodies against the particular snake venom (antigen), essentially destroying the venom so that its particular physiological effects on body cells and tissues cannot continue.*

## In-Class Activities

**(lesson ideas, including labs & demonstrations)**

1. The basics of an antibody-antigen that is involved when using snake antivenom reactions can be studied through the use of a blood-typing kit available from the usual school science supply companies. Today, a synthetic blood rather than real blood from students (that used to be possible in pre-HIV days!) is used to carry out the reactions that identify the different blood types through antibody-antigen reactions (done on a microscale level). Students can see the clumping reaction that occurs in this type of reaction. The kits always contain complete instructions.

* One source for these kits is found at Ward’s Scientific, at <https://www.wardsci.com/store/catalog/product.jsp?catalog_number=360034>.
* A second supplier of blood testing kits is from Flinn Scientific at <http://www.flinnsci.com/store/Scripts/prodView.asp?idproduct=18824>.
* A third source is from Carolina Biologic at <http://www.carolina.com/blood-typing/carolina-abo-rh-blood-typing-with-synthetic-blood-kit/FAM_700101.pr?catId=10466&mCat=10337&sCat=10415&ssCat=&question>.

1. Software from HHMI provides a lab interactive simulation that takes a student through the blood-typing process: <http://www.hhmi.org/biointeractive/immunology-virtual-lab>.

And this Web page provides a pdf worksheet for students to us while going through the HHMI simulation: <http://www.hinsdale86.org/staff/kgabric/labsOnline/Immunology%20virtual%20labAnswers.pdf>.

1. Here is a third option for the blood-typing activity, an online game-type simulation for blood-typing accident victims. This game is part of the Nobel Prize Web site, and it also has a competition phase, with high score listed! (<http://www.nobelprize.org/educational/medicine/bloodtypinggame/index.html>)
2. Students could be shown the methods by which snake venom is collected (always of interest to teenagers!) by referring to this video: <https://www.youtube.com/watch?v=inrbYyfUZX8>.
3. A very interesting and understandable lecture from the Howard Hughes Medical Institute (HHMI) done specifically for students (they are a select audience through an HHMI outreach program) can be found here: <http://www.hhmi.org/biointeractive/venoms-drugs>. The lecture features one of the principle investigators, Dr. B.M. Olivera, and his research into how the venom of the cone snail works on the nervous system and how it has been developed into a useful pain-killing medicine (Ziconotide, mentioned in the *ChemMatters* article above). This lecture clearly illustrates how a researcher’s thinking leads to useful information. Dr. Olivera presents, with illustrations, a very audience-friendly lecture geared to students. A student Q & A session with Dr. Olivera taken from the lecture is shown at <http://www.hhmi.org/biointeractive/qa-cone-snails>.
4. Since so much of the discussion about venom and its derivative compounds centers around protein structure and synthesis, students can participate by simulating the synthesis of a general protein structure utilizing RNA and specific amino acids which follow DNA-dictated instructions (code). It is the quickest way to understand the connections between DNA (genetics), RNA, amino acids and protein synthesis. Two complementary Web-based activities that should be used in this particular order are found at <https://www.wisc-online.com/learn/natural-science/life-science/ap1302/protein-synthesis> and <http://sepuplhs.org/high/sgi/teachers/genetics_act16_sim.html>. The first activity illustrates the protein synthesis in a cell, and the second activity video (interactive) has the students synthesize a portion of a protein by moving amino acids into the correct binding position on the RNA surface.

# Out-of-class Activities and Projects

**(student research, class projects)**

1. If students have an interest in both genetic programming of a bacterium and the use of color indicators in specific cells (related to the chlorotoxin in the *ChemMatters* Haines venom article used to mark cancer cells), there are complete classroom kits with instructions for carrying out the genetic manipulation with visible results. Students can create their own fluorescent cells similar to those of chlorotoxin mentioned in the Haines article using kits available for school science programs. The kits illustrate how genetic material can be transferred from one type of cell to another and continue to operate in the new host cell. This is a common practice these days and is used to reprogram bacteria and yeast cells to produce a particular molecule that is vital to a drug’s or vaccine’s function. As mentioned in the Haines article, certain drugs based on venom components are mass produced through what is called fermentation, using the re-engineered bacteria or yeast as synthesizers of the drug. One activity kit for transforming a bacterium (*Escherischia coli*) into a glowing cell by transferring a particular gene from a glowing bacterium, *Vibrio fischerican* can be purchased from a school supply company (Carolina) at <http://www.carolina.com/transformation-dna-transfer-kits/glow-in-the-dark-transformation-teacher-demo-kit-with-prepaid-coupon/211086.pr?question>.

A second kit from the same company provides students with materials and procedures to transfer some genetic material (Green Fluorescent Protein, GFP) from a jelly fish into *E. coli* bacteria that is then able to produce fluorescence under UV light. Refer to <http://www.carolina.com/transformation-dna-transfer-kits/ap-biology-lab-6-molecular-biology-module-1-transformation-8-station-kit-with-prepaid-coupon/211082.pr?catId=&mCat=&sCat>.

# References

**(non-Web-based information sources)**



**30 Years of *ChemMatters***

Available Now!

**The references below can be found on the *ChemMatters* 30-year DVD (which includes all articles published during the years 1983 through April 2013 and all available Teacher’s Guides, beginning February 1990). The DVD is available from the American Chemical Society for $42 (or $135 for a site/school license) at this site:** [http://ww.acs.org/chemmatters](http://www.acs.org/chemmatters)**. Scroll about half way down the page and click on the *ChemMatters* DVD image at the right of the screen to order or to get more information.**

**Selected articles and the complete set of Teacher’s Guides for all issues from the past three years are available free online on the same Web site, above. Simply access the link and click on the “Past Issues” button directly below the “M” in the *ChemMatters* logo at the top of the Web page.**

A very detailed article about the application of bioluminescence in research into cell function, including the use of the bioluminescence to mark cells or to identify locations of intracellular chemical activity is found in the December, 2008 *ChemMatters*. (Zaja, L. Glowing Proteins With Promising Biological and Medical Applications. *ChemMatters* **2008,** *26* (4), pp   
12–14.

The December 2008 Teacher’s Guide that accompanies the glowing proteins article cited above provides a good assortment of references on both bioluminescence and chemiluminescence, as well as some lab activities related to both topics.

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A 2014 article from *Scientific American* discusses the various types of chronic pain, many of which defy treatment. New insights into the causes of pain, particularly at the molecular level, suggest some new interventionist strategies to alleviate the pain. This includes using drugs, some of them from venom, that interact with the different ion channels. The article has some useful drawings and charts. Refer to Sutherland, S. Pain That Won’t Quit. *Scientific American*, **2014**, *311* (6), pp 60–67.

# Web Sites for Additional Information

**(Web-based information sources)**

**More sites on** **overall discussion of the use of venom-derived molecules in medicine and the techniques for collecting snake venom**

The reference in the Haines venom article for a *National Geographic* article on research into venom extracts for use in medicine is a good supplement to the Haines article, containing many more specific examples of various venom sources for developing particular medicines. It can be accessed at <http://ngm.nationalgeographic.com/2013/02/125-venom/holland-text>.

An interesting video (1:13) showing the milking of snakes for their venom can be viewed at <https://www.youtube.com/watch?v=4WvnjCkLbvY>. A demonstration also shows the effect of the venom on clotted blood. Note that the narration is not in English, but that doesn’t detract from the viewing.

The details for collecting snake venom can be found at this Web site: <http://www.tamuk.edu/nntrc/facilities/venom_collection.html>. This is a commercial operation run at Texas A & M University in Kingsville. They have a price list for all the different venoms collected.

A concise collection of descriptions about the medical uses of different venoms (different animal sources) can be found at the PBS Web site <http://www.pbs.org/wgbh/nova/body/venoms-healing-bite.html>.

**More sites on** **using antibody-antigen mechanisms for delivering site-specific drugs**

An article from *Scientific American* describes the techniques for designing drug-delivering techniques, utilizing antibody-antigen interactions in order to target specific sites for delivery. The article also points out the difficulties in using this approach. Refer to:

<http://blogs.scientificamerican.com/guest-blog/2013/07/04/antibody-drug-conjugates-and-cancer-treatment-making-smart-bombs-smarter/>.

More examples of nanoparticle delivery techniques can be found at these two Web sites:

* This Web site describes many approaches for dealing with cancer. The use of nanotechnology enhances several aspects of either detecting and eliminating cancer cells before they form tumors, or destroying cancer cells with minimum damage to healthy tissue. (<http://www.google.com/url?q=http%3A%2F%2Fwww.understandingnano.com%2Fcancer-treatment-nanotechnology.html&sa=D&sntz=1&usg=AFQjCNEHTCltCE7omN4k1ERRGRgsm--skw>)
* This Web site provides examples of how nanotechnology is used not only in cancer treatment but also in a number of other therapeutic areas: heart disease; delivering drugs needed for treating other conditions (e.g., diabetes, glaucoma, and autoimmune diseases, including multiple sclerosis, and brain disorders); and penetrating the mucous that covers lung tissue in order to deposit therapeutic drugs on the lung surface. (<http://www.understandingnano.com/nanotechnology-drug-delivery.html>)

**More sites on GFP—green fluorescent protein**

The history behind the discovery and isolation of the genes that control the production and activation of the green fluorescent protein (GFP) is found at <http://www.brighthub.com/science/genetics/articles/50421.aspx>. These genes are widely used as genetic markers, similar to the chlorotoxin mentioned in the Haines article. Some chemistry related to the function of this protein is found in this article.

**More sites on elucidating the structure and function of potassium channels**

Determining the structure of potassium channels and how they operate was the work of Roderick MacKinnon for which he won the Chemistry Nobel prize (2003). Being able to “see” the structure of these channels involved the use of X-ray crystallography. An elaborate description of what went into this research by McKinnon himself is found at <http://www.hhmi.org/news/mackinnon-wins-2003-nobel-prize-chemistry>.

Another interview article with MacKinnon which may be of interest to students in terms of how research scientists think and do, so to speak, is found in this *Scientific American* article, <http://www.scientificamerican.com/article/interview-with-roderick-m/>. It also contains additional information on the workings of potassium channels.

A related article and video about how these potassium channels work are found at <https://www.quantamagazine.org/20140220-new-clues-to-pain-sensor/>.

**More sites on** **spider and centipede venom research**

The most recent research into proteins from spider venom for controlling pain is “… focused on the world's 45,000 species of spiders, many of which kill their prey with venoms that contain hundreds—or even thousands—of protein molecules. Some of these molecules block nerve activity. Refer to <http://www.sciencedaily.com/releases/2015/03/150304075410.htm>.

Promising work on a peptide derived from the venom of the centipede that could control pain in the peripheral nervous system (outside the spinal cord), preferable to the peptide from cone snail venom that has to be injected into the spinal cord, is discussed in the following reference:

<http://medicalxpress.com/news/2013-09-centipede-venom-class-pain-drug.html#nRlv>.

# More Web Sites on Teacher Information and Lesson Plans

**(sites geared specifically to teachers)**

The Howard Hughes Medical Institute’s Web site has many user-friendly articles, references, videos (including lectures geared toward students by research scientists), class activities, and a quarterly scientific publication for teachers (paper and on-line). Refer to the following site, and enjoy exploring: [www.hhmi.org](http://www.hhmi.org).