Frog protein may mitigate dangers posed by toxic marine microbes

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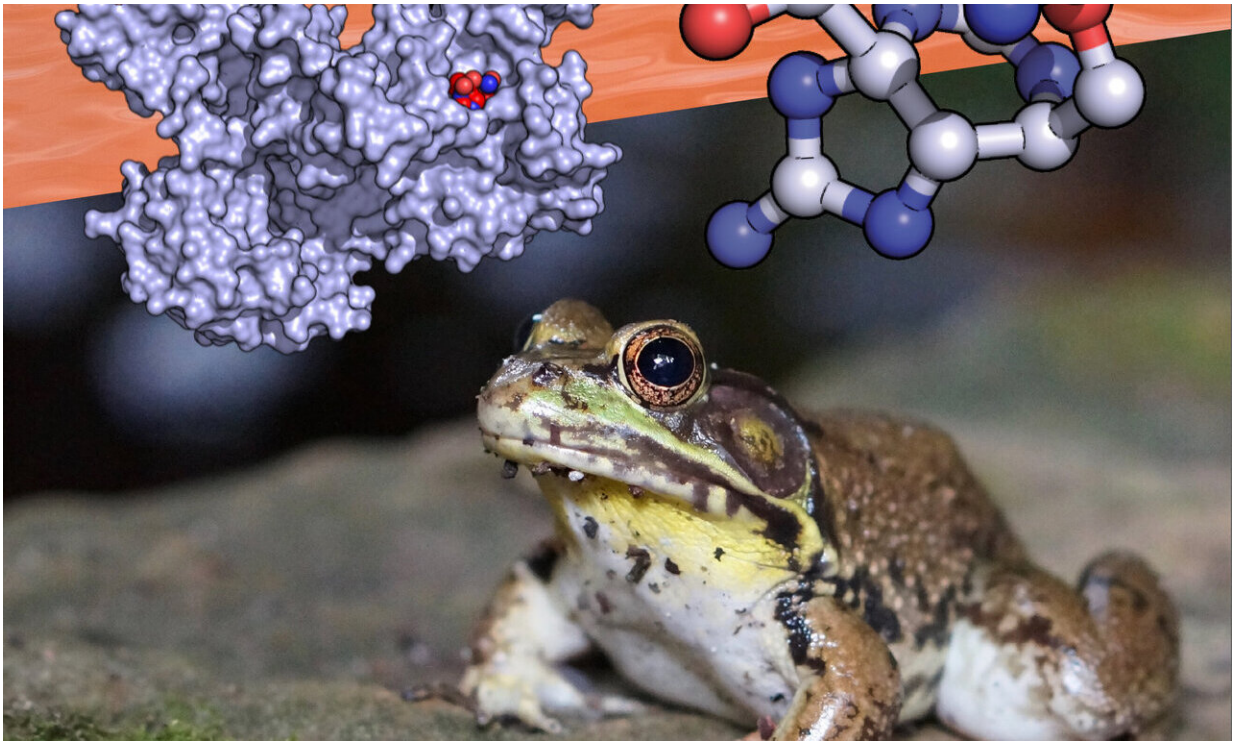

Molecular structure of American bullfrog saxiphilin reveals the blueprint of a 'molecular sponge' for capturing saxitoxin, a lethal paralytic toxin produced by red tide. Credit: Daniel L. Minor, Jr., Ph.D. and Deborah Stalford

A new study from UC San Francisco suggests that a protein found in the common bullfrog may one day be used to detect and neutralize a poisonous compound produced by red tides and other harmful algal

blooms. The discovery comes as these waterborne toxic events are becoming increasingly common, a consequence of climate change making the world's oceans more hospitable to the microbes responsible for these formerly infrequent flare-ups.

Aquatic microbes manufacture an array of toxic compounds, but few are as formidable as the nerve agent saxitoxin, often found in algal blooms. Though not a household name like cyanide, saxitoxin is far more potent—it's deadly at doses a thousand times smaller than a lethal dose of cyanide. And because it accumulates in filter-feeding shellfish, saxitoxin can work its way up the food chain and land on our dinner tables.

"Saxitoxin is among the most lethal natural poisons and is the only marine toxin that has been declared a chemical weapon," said Daniel L. Minor Jr., Ph.D., senior author of the new study and professor at UCSF's Cardiovascular Research Institute.

The research, a collaboration between Minor's lab and the laboratory of Stanford University Chemistry Professor Justin Du Bois, Ph.D., was published June 19 in *Science Advances* and provides the first high-resolution molecular structures of saxiphilin, a remarkable protein that is thought to render bullfrogs resistant to the neurotoxic effects of saxitoxin.

These structures—detailed renderings that depict saxiphilin both by itself and bound to saxitoxin—help explain how the protein confers this protective benefit and offer key insights into the evolutionary origins of toxin resistance. These findings provide a more thorough understanding of saxiphilin that will allow scientists to begin exploring ways to repurpose the protein for use as a tool to detect the toxin, both in the environment and in the body, and as an antitoxin therapy.

Frogs Are Resistant to Red Tide Toxin That Kills Victims by Paralyzing Nerve Cells

When ingested by humans, saxitoxin disrupts nerve signaling and can lead to a potentially fatal condition known as paralytic shellfish poisoning. Without immediate medical attention, the muscles that control breathing are rapidly incapacitated, leading to death by asphyxiation.

In the wild, the effects can be even more extreme. During red tides and other saxitoxin outbreaks, the lifeless carcasses of aquatic animals—from fish and turtles to whales and dolphins—are often found washed ashore by the hundreds. But you won't find frogs among the dead and dying.

Frogs are unusually saxitoxin resistant, a phenomenon first observed by Hermann Sommer, Ph.D., a researcher at the George Williams Hooper Foundation at UCSF from 1924 until his death in 1950. From 1928 to 1932, Sommer collected bivalves along the San Francisco coast and isolated what was then called "mussel poison." He tested its effects on lab animals and found that frogs didn't exhibit severe symptoms until they were given at least 15 times the lethal dose for mice. At the time, however, Sommer couldn't account for this result.

Later efforts by other researchers showed that saxitoxin's main target is a class of proteins known as voltage-gated sodium channels. These proteins, which are embedded in the surface of nerve and muscle cells, control the flow of electrically charged particles in and out of the cell.

"The interaction of the toxin with voltage-gated sodium channels is the reason why saxitoxin is so poisonous, as this interaction blocks electrical signals in nerves," Minor explained.

Though scientists understood this mechanism of neurotoxicity—and also knew that saxiphilin could somehow counteract these effects—Minor and colleagues had to deduce saxiphilin's molecular structure before they were able to explain exactly how the protein protected frogs from saxitoxin poisoning.

Molecular Architecture Explains Protein's Antitoxic Properties

To do this, the researchers turned to a technique known as X-ray crystallography, which is among the most common methods used for solving the structures of complex biomolecules. It was used in the 1950s to determine the structure of DNA, and remains the gold standard in structural biology to this day. And it was X-ray crystallography that provided the first saxiphilin structures—maps that detail the three-dimensional arrangement of the atoms that comprise the protein.

With these biochemical blueprints in hand, Minor and colleagues were able to account for the bullfrog's remarkable saxitoxin resistance. The structures revealed a catcher's mitt-like "pocket" that ensnares saxitoxin in a web of powerful electrostatic interactions. Unable to escape from this electric vise, saxitoxin is no longer free to bind to voltage-gated sodium channels and disrupt nerve signaling.

"Saxiphilin acts as a 'toxin sponge' that sops up and sequesters this deadly microbial poison," said Minor.

Saxiphilin Ancestor Reveals How Proteins Evolve Novel Anti-Toxic Functions

The researchers also noticed an uncanny structural similarity between saxiphilin and transferrins, a family of proteins that transport iron to red

blood cells from sites in the body where iron is absorbed or stored. When superimposed, the two proteins look nearly identical. But a closer inspection of their structures revealed key differences that explain their divergent function and also suggests that the reason they look so much alike is because saxiphilin evolved from a member of the transferrin family.

Transferrins have two iron binding sites, one of which abuts what Minor and colleagues refer to as a "proto-pocket." Though this proto-pocket is too small to bind saxitoxin—and isn't known to bind any other molecules—the scientists were able to show that replacing a small handful of positively charged amino acids with negatively charged amino acids transformed this functionless structural feature into a life-saving saxitoxin sponge. But they also discovered that the molecular architecture responsible for saxitoxin binding isn't unique to saxiphilin.

"Our findings uncover a remarkable convergence in how saxiphilin and voltage-gated sodium channels recognize the toxin. Both proteins share the same general blueprint for saxitoxin recognition," Minor said.

When Minor and colleagues compared their saxiphilin structures against existing structures that show saxitoxin bound to voltage-gated sodium channels, they discovered that these two classes of protein share a common architecture that facilitates their interaction with saxitoxin, even though they're otherwise structurally and evolutionarily unrelated.

An analysis of protein sequences from other animals revealed that similar saxitoxin binding pockets also appear in proteins from distantly related species, including the High Himalaya frog. This finding suggests that a common saxitoxin binding pocket evolved multiple times in the course of evolutionary history, though scientists are not entirely sure why these proteins evolved in the first place.

"This work is an important first step to understanding how organisms can evolve resistance to toxic environments," Minor said, also noting that the research has important practical applications that may be of particular interest to Bay Area residents.

"This problem is increasing with climate change and is a serious public health and commercial fishing hazard. Red tide warnings have closed California fisheries multiple times in the past few years and affect San Francisco, Marin and San Mateo counties. Our efforts may lead to new ways to detect saxitoxin and counter saxitoxin poisoning, for which there are currently no approved treatments."

More information: "Structure of the saxiphilin:saxitoxin (STX) complex reveals a convergent molecular recognition strategy for paralytic toxins" *Science Advances* (2019). advances.sciencemag.org/content/5/6/eaax2650

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