Researchers use snake venom to solve structure of muscle protein

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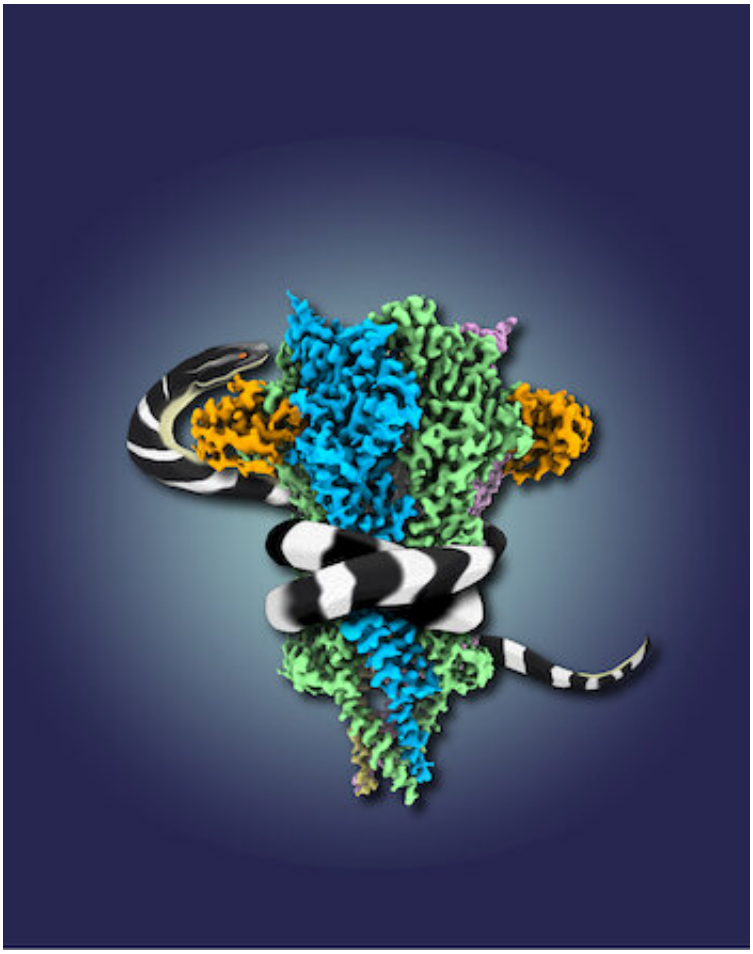

First high-resolution structure of native muscle-type nicotinic receptor purified from the electric organ of the Pacific ray and surrounded by a banded krait. A neurotoxin from the krait's venom was used in the study. Credit: UTSW

Researchers at UT Southwestern Medical Center have uncovered the detailed shape of a key protein involved in muscle contraction. The report, published today in *Neuron*, may lead to improved understanding of muscle-weakening genetic conditions called congenital myasthenic syndromes (CMS).

The protein sits on the surface of nerve cells that connect to muscles and is integral to triggering the muscle cell to contract just milliseconds after instructions are sent through the spinal cord. Called a nicotinic receptor, it has been a challenge to study because it sits in the cell's membrane.

"The nicotinic receptor at the neuromuscular junction has been a target of interest for over a century. It was the first ion channel to be purified, the first to have its genes cloned, and the first to be imaged by an electron microscope," says Ryan Hibbs, Ph.D., associate professor of Neuroscience and Biophysics at UTSW and a corresponding author of the study.

Many groups had tried to determine the receptor's structure using an earlier technology called X-ray crystallography as well as first generation cryogenic electron microscopes (cryo-EM) but they were only able to obtain low-resolution images, he adds.

Normally the nicotinic receptor is activated by a molecule called acetylcholine. However, the nicotinic receptor is also the target of various venoms that cause muscle paralysis.

So the team used this to their advantage to isolate enough of the receptor protein to study its shape and structure. They mixed the toxin from snake venom with fish tissue known to contain high amounts of the receptor protein.

The team then flash froze the receptor bound to the toxin and used the

rapidly evolving technique of cryo-electron microscopy to uncover the shape of the structure. Before recent developments in cryo-EM, the only method by which to solve protein structures like the nicotinic receptor was X-ray crystallography, which involved slowly growing crystals of proteins. But proteins that sit in membranes usually do not crystallize well.

"This never could have been done using X-ray crystallography—hundreds of researchers had attempted it. The new microscopes allowed us to get to a very high resolution near the atomic level. At that resolution, we can precisely make out the positions of most of more than 2,000 amino acids that make up the receptor protein," Hibbs says. "The results were stunning. They revealed, at a very fine level of detail, the 3-D architecture of the receptor, with two toxin molecules bound to it exactly where we know the much smaller acetylcholine binds."

The results showed how the toxin blocks acetylcholine by competing for the same binding site, paralyzing the receptor in the closed configuration and preventing the flow of electrochemical messages. The structure also reveals why the toxin binds so tightly and selectively to the receptor and how the venom paralyzes prey, including humans, who are unfortunate enough to get bitten by the snake.

"We learned a tremendous amount from the new 3-D structure beyond how the toxin works to poison the receptor," Hibbs says. Lead author Md. Mahfuzur Rahman, Ph.D., a postdoctoral researcher in the Hibbs lab, adds that the nicotinic acetylcholine neuromuscular receptor is a therapeutic target for several genetic diseases that cause muscle weakness, which the team also investigated.

"Here we mapped the mutations related to CMS to three principal regions on the receptor structure. This new structural information sheds

light on how mutations in those areas result in muscle-weakening syndromes," Rahman says.

More information: Md. Mahfuzur Rahman et al, Structure of the Native Muscle-type Nicotinic Receptor and Inhibition by Snake Venom Toxins, *Neuron* (2020). DOI: 10.1016/j.neuron.2020.03.012

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