in a general mode that alters the conformation of the CRD, Q29 may offer an additional advantage in reducing the likelihood for resistance to rise. However, the $IC_{50}$ of Q29 is above the micromolar range, making it far too weak to directly progress to the next stages of drug development. Nevertheless, the identification of Q29 opened a new avenue for further exploration, perhaps through medicinal chemical refinement or compound screens designed to specifically block the covalent cholesterol modification.

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## DECLARATION OF INTERESTS

The authors declare no competing interests.

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# STX-bpc: "Brightening" the path to neuronal inhibition

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**In this issue of *Cell Chemical Biology*, Elleman et al.[1] introduce a transformative chemical approach to control neuronal activity with high spatial and temporal resolution. The authors present STX-bpc, a potent neurotoxin that naturally inhibits voltage-gated sodium channels ($Na_V$s), complementing available optogenetic methods for manipulating neuronal activity, cellular communication, and behavior.**

Precise manipulation of neuronal activity is a powerful approach in neuroscience, helping to dissect the roles of neurons in the functional organization of neural circuits and their impacts on diverse behaviors. Genetically encoded actuators such as light-gated ion channels and pumps have been widely used to control neuronal activities with high spatial and temporal resolution which has revolutionized neuroscience research. While chemogenetic tools, which typically operate over broader temporal and spatial scales, serve as a valuable adjunct to optogenetics. Both optogenetics and chemogentics rely on the expression of exogenous proteins—be they light sensitive opsins or G-protein-coupled receptors (GPCRs).[2–5] The process of genetic manipulation, however, can be fraught with challenges or excessive time requirements, particularly in certain species that are less amenable to such modifications. In light of these limitations, optochemical approaches emerge as a viable and complementary technique. They offer a means to modulate neuronal

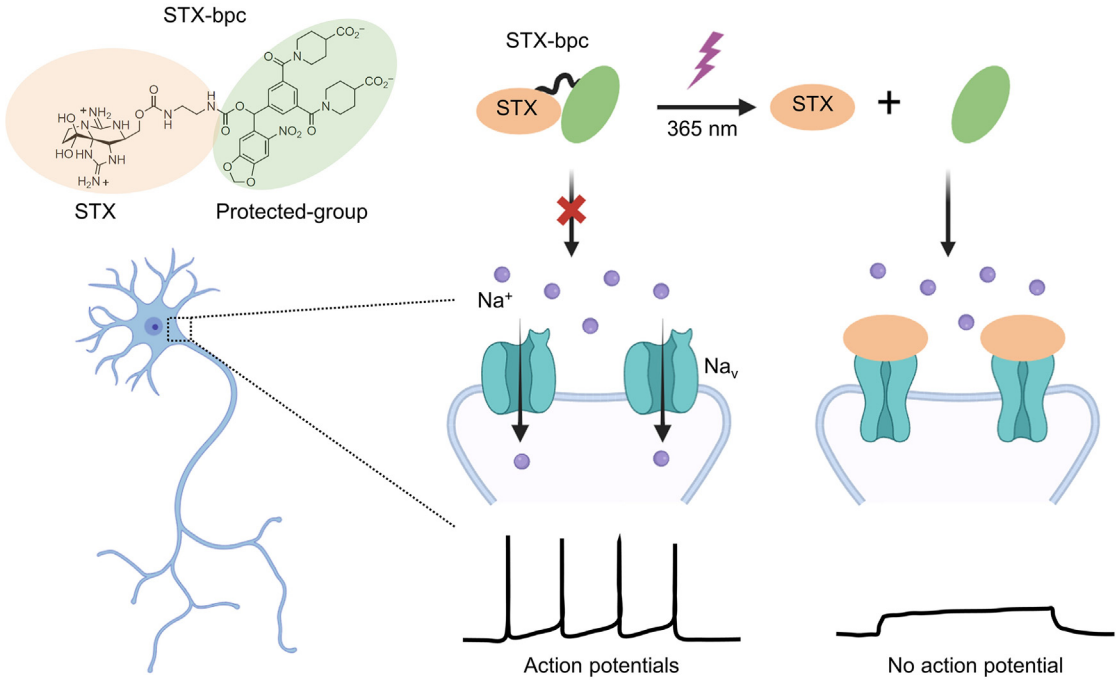


**Figure 1. The principle of photocaged STX for blocking neuronal activities**

STX-bpc, a photocaged derivative of saxitoxin, cannot bind to voltage-gated sodium channels ($Na_V$s) in its inactive form. Upon UV light (365 nm) illumination, the active STX is released, binds to $Na_V$s, and blocks the channel, preventing action potentials.

activity through photo-deprotection or photo-isomerization upon light exposure, circumventing the need for genetic engineering. This innovative method presents a promising avenue for investigating the neural substrates of behavior and cognition without the constraints associated with genetic manipulation.

In this study, Elleman et al.[1] introduce an optochemical approach to achieve rapid and reversible silencing of neuronal action potentials (APs) with a modified form of saxitoxin (STX), a potent blocker of voltage-gated sodium channels ($Na_V$s) (Figure 1). The key innovation lies in the development of a photocaged derivative of STX (STX-bpc) that remains inactive until exposed to light. This offers a non-invasive, genetically independent alternative for manipulating neuronal activity with high precision both *in vitro* and *in vivo*.

Previously, Elleman et al. developed coumarin-caged STXs, but these compounds exhibited low sensitivity and were toxic to neurons due to limited uncaging efficiency and an unsatisfied binding affinity to the $Na_v$s, being only about 20 times less potent than active STX.[6] To address these issues, they introduced a sterically large and negatively charged moiety to the photocleavable protecting group, reducing the affinity of the caged STX to $Na_V$s in the absence of light.[7] After rigorous characterization, they identified STX-bpc, a compound 270 times less potent than STX but still capable of efficiently blocking $Na_V$s at non-toxic concentrations. The efficiency of the $Na_V$ block could be fine tuned by varying STX-bpc concentrations, light intensity, and illumination duration.

The author demonstrated the utility of the STX-bpc in blocking neuronal activities in dissociated rat hippocampal neurons, dissociated rat dorsal root ganglia (DRG), mouse cortical brain slices, and larval zebrafish. In dissociated rat hippocampal neurons, exposure to a single 5-ms laser pulse was sufficient to cleave the protecting group and release active STX to bind $Na_V$s and block a large portion of $Na_V$s currents. The AP firing rate decreased in a concentration-dependent manner with photolysis of STX-bpc, and uncaging of 500 nM STX-bpc blocked all APs. These data demonstrate that photosensitive STX-bpc could be used to modulate APs with precision in hippocampal neurons through the rapid and selective block of $Na_V$s. In rat DRG cells, uncaged STX-bpc showed high efficiency in blocking APs, with negligible effects on AP shape and amplitude. The utility of STX-bpc for controlling neuronal activities in tissue was demonstrated in mouse acute cortical brain slices. The precise application of light allowed for specific layer-targeted inhibition of neuronal populations, inhibiting different subpopulations of $Na_V$s within the axon and soma dendritic compartments with similar efficiency. Photo-unprotected STX-bpc quickly terminated the AP firing from different cell types, such as regular spiking neurons and fast spiking neurons, within seconds of LED light illumination. The silencing efficiency was comparable between different cell types, showcasing that the inhibition efficiency of STX-bpc is similar through diverse subcellular compartments and varying cell types. Finally, the utility of STX-bpc in live animals was demonstrated using larval zebrafish, a model organism frequently used in neuroscience research. By directing light to specific brain regions such as the brainstem, researchers were able to inhibit neuronal activity in targeted areas, leading to observable changes in swimming behavior regulated

by the brainstem. The STX-bpc-induced neuronal silencing in zebrafish swimming behavior was repeatable, presumably due to the dissociation of the active toxin. With focal illumination on one half of the zebrafish brainstem, the STX-bpc precisely silenced the ipsilateral sensory neurons related to swimming behavior, causing the tail to bend to the contralateral side. Collectively, focal uncaging realizes precise neuronal silencing and rapid, reversible control of zebrafish behavior.

Compared with other widely used approaches, STX-bpc offers several advantages:

(1) Genetically free: Unlike optogenetics, which requires the introduction of light-sensitive proteins through genetic modification, STX-bpc can be used without the expression of exogenous proteins in the target cells. This makes it suitable for use in a broader range of organisms, especially those that are difficult to make transgenic animals or infected with virus.
(2) Reversibility: The effects of STX-bpc are reversible, allowing for transient and repeated inhibition of neuronal activity. This reversibility is crucial for studying dynamic processes in neural circuits and understanding how temporary changes in activity can lead to lasting behavioral outcomes.
(3) Precision: The spatial and temporal precision of STX-bpc is comparable to optogenetics and much better than pharmacogenetic methods. The ability to activate the toxin with light ensures that only the targeted neurons are affected, minimizing off-target effects and preserving the integrity of surrounding neural tissue.

While the study presents a compelling case for the use of STX-bpc, it also acknowledges potential limitations such as the need for UV light for uncaging, which can cause tissue damage, especially with prolonged neuronal silencing. Additionally, there are variable washout times of STX-bpc across different tissues, making it difficult to estimate the working time of this toxin across different organisms. Despite these considerations, the benefits of STX-bpc outweigh its drawbacks, particularly given its lack of need for genetic manipulation and the rapid response time following irradiation.

Elleman et al.'s study presents a significant advancement in the field of neurobiology by introducing a novel optochemical method for the precise, rapid, and reversible silencing of neuronal activity. The development of STX-bpc provides a powerful tool for manipulating neuronal activity without the need for genetic modification. These advantages make it a valuable addition to the neuroscientist's toolkit, with broad applications in research on neural activity, behavior, and neurological diseases.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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