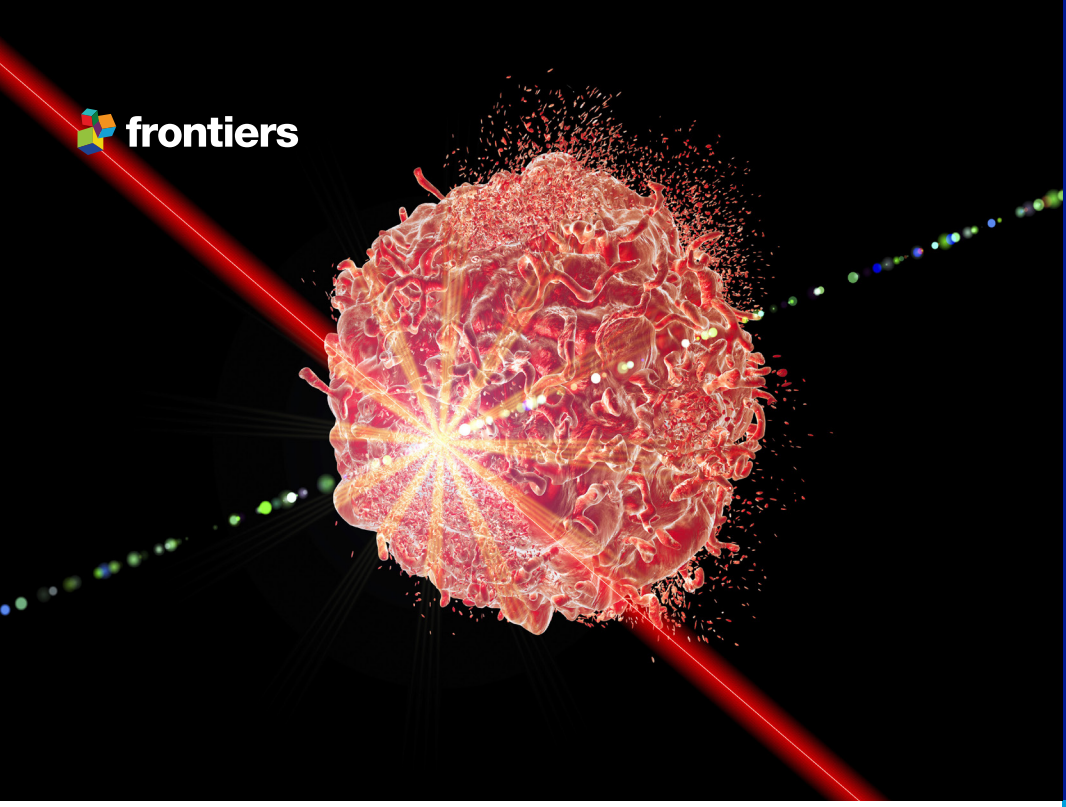

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Targeted covalent photoswitch for two-photon control of endogenous receptors

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+Equivalent contribution

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Ramona, S., Maleeva, G., Sortino, R., Pons-Allés, S., Ramos-Guerra, C., Matera, C., Gorostiza, P. Targeted covalent photoswitch for two-photon control of endogenous receptors.

The study of intact cells and their signaling circuits with light requires a stimulation strategy that is focused, deeply penetrating, and that does not damage them. Implanted optic fibers, light emitting diodes, and luminescent materials operated externally with tissue-penetrating infrared (IR) light are invasive or limited by light attenuation around the illumination point.

To overcome these barriers, two-photon pharmacology takes advantage of femtosecond-pulsed IR laser light to produce deep and spatiotemporally precise cellular stimulation using specially designed photoswitchable drugs. Compounds that can be covalently tethered to the target neuroreceptor perform particularly well. However, the tethered photoswitches reported to date require mutagenesis of the target protein, which prevents using

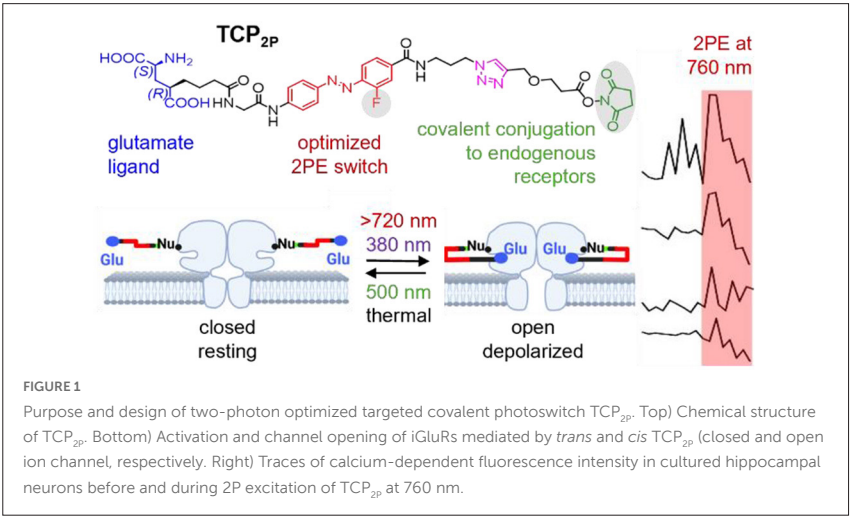

FIGURE 1
Purpose and design of two-photon optimized targeted covalent photoswitch TCP_{2P}. Top) Chemical structure of TCP_{2P}. Bottom) Activation and channel opening of iGluRs mediated by *trans* and *cis* TCP_{2P} (closed and open ion channel, respectively. Right) Traces of calcium-dependent fluorescence intensity in cultured hippocampal neurons before and during 2P excitation of TCP_{2P} at 760 nm.

photopharmacology to stimulate the nervous system in wildtype animals. Here, we report the first two-photon optimized targeted covalent photoswitch (TCP_{2P}) that combines the efficient two-photon isomerization of *ortho* fluoro substituted azobenzene with the ability to conjugate to nucleophilic residues of endogenous proteins (AMPA and kainate ionotropic glutamate receptors in neurons). TCP_{2P} is readily obtained by click coupling of two precursor compounds prior to use and after simple incubation it enables controlling neuronal activity at one and two-photon excitation up to 800 nm without genetic modifications (Figure 1).

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