NEUROSCIENCE

Expanding the optogenetics toolkit

Flipping opsins within the membrane diversifies the array of available optogenetics tools.

Optogenetics has been instrumental for the dissection of functional neural circuits. As this branch of neuroscience flourishes, and as experimental designs become increasingly sophisticated, the need for new opsins—light-activated proteins that can be used to excite or inhibit specific neuronal populations—also grows. Previous efforts have relied on either the discovery of naturally occurring opsin variants with unique features through genome mining, or the engineering of opsins to achieve certain desired properties. Now, researchers at the Janelia Research Campus of the Howard Hughes Medical Institute, led by Joshua Dudman and Alla Karpova, have developed a new protein-engineering approach that could in principle double the number of viable tools for optogenetics.

Taking inspiration from evolution, the researchers wondered whether topological inversion—flipping a protein inside-out within the membrane—could generate opsin variants with new properties. They designed a leader sequence containing the signal peptide and the transmembrane domain of neurexin 1B-δ, and they packed in a few more tweaks to further support its desired orientation. They then fused the leader sequence and a well-characterized opsin, ChR2 E123T/T159C. This effectively flipped the opsin within the membrane. Strikingly, the opsin was transformed from an optogenetic activator to a potent inhibitor that functions as a light-activated, nonselective cation pump. They dubbed this new variant 'full-length inversion of ChR' (FLInChR).

FLInChR suppressed the activity of SNr GABAergic neurons in brain slices. In vivo, FLInChR was able to inhibit SNr projection neurons and modulate animal behaviors. Notably, the extent of inhibition elicited by FLInChR was on par with that observed with ArchT, a commonly used optogenetic inhibitor.

It is worth noting that nonselective cation pumping has not been previously achieved through directed molecular engineering. This topological engineering approach could help to create new optogenetic tools for neuroscience research.

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Research papers
Brown, J. et al. Expanding the optogenetics toolkit by topological inversion of rhodopsins. *Cell* **175**, 1131–1140 (2018).