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Structural basis for color tuning and ion selectivity in potassium-selective channelrhodopsins

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Abstract

Optogenetics is a groundbreaking experimental technique that allows scientists to control the membrane potential of cells, particularly neurons, using light. This method relies on light-responsive proteins called channelrhodopsins (ChRs), which are found in microorganisms and function as light-activated ion channels. Optogenetics has profoundly impacted neuroscience by enabling precise manipulation of neural circuits in the brain.

To advance optogenetic experiments and better understand brain function, researchers have discovered and engineered various types of ChRs with diverse properties. However, most ChRs developed so far fall into two categories based on the ions they transport: non-selective cation channels, which activate neurons, and Cl^- channels, which inhibit them. While Cl^- channels are useful for suppressing neuronal activity, their effectiveness is limited because the balance of Cl^- inside and outside cells can fluctuate.

In 2021, scientists made a significant breakthrough by discovering potassium channelrhodopsins (KCRs), a new type of ChR that selectively transports K^+. KCRs are considered ideal tools for inhibiting neurons because they offer more stable and reliable control compared to Cl^- channels. Unlike traditional potassium channels, KCRs have entirely different amino acid sequences and structural features, and the mechanism by which they specifically select K^+ ions was previously unknown.

In our research, we used cryo-electron microscopy to determine the structures of two newly discovered KCRs, HcKCR1 and HcKCR2. By combining these structural insights with electrophysiological and

computational analyses, we uncovered the molecular basis for why HcKCR1 and HcKCR2 absorb different wavelengths of light and how they selectively transport K^+. Additionally, we developed a mutant version of KCR, named KALI, which shows enhanced selectivity for K^+ and outperforms existing inhibitory optogenetic tools.

Our findings not only deepen the understanding of how KCRs function at a molecular level but also provide improved tools for neuroscientific research, enabling more precise control over neuronal activity.