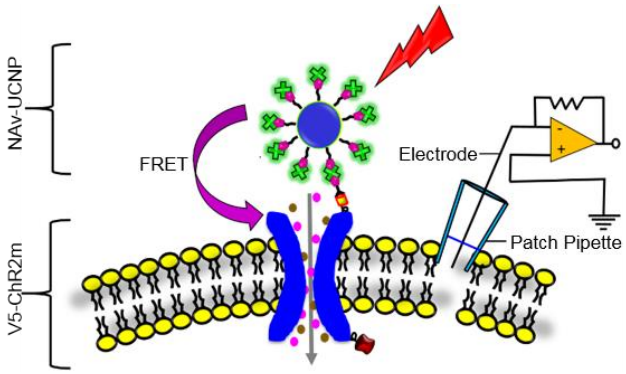
Near-Infrared Optogenetic Activation of Channelrhodopsin Expressed in Living Cells via Upconverting Nanoparticles with Target Specificity

Abstract

Optogenetics is an innovative technology now widely adopted by researchers in different fields of the biological sciences. However, most proteins adopted in optogenetics are excited by ultra-violet or visible light that has a weak tissue penetration capability. Upconverting nanoparticles (UCNPs), which transform near-infrared (NIR) light to short-wavelength emission, can help address this issue. To improve optogenetic performance, we enhance the target selectivity for optogenetic controls by specifically conjugating the UCNPs with light-sensitive proteins at a molecular level, which shortens the distance as well as enhances the efficiency of energy transfer. We tagged V5 epitope to the extracellular N-terminal of channelrhodopsin-2 with a mCherry conjugated at the intracellular C-terminal (V5-ChR2m) and then bound NeutrAvidin-functionalized UCNPs (NAv-UCNPs) to the V5-ChR2m via a biotinylated antibody against V5. The results showed NAv-UCNPs bound to the plasma membranes of cells expressing V5-ChR2m, but not ChR2m without the V5 epitope. Under NIR illumination, the NIR-upconverted blue illumination from UCNP induced an inward cation current and elevated the intracellular Ca^{2+} concentration in the live cells expressing V5-ChR2m bound with NAv-UCNPs. The fluorescence resonance energy transfer (FRET) from the excited UCNP to the V5-ChR2m was confirmed by fluorescence lifetime imaging measurements. Our results demonstrate that when membrane channelrhodopsin is specifically anchored by UCNPs in the molecular level, the energy transfer via irradiance and FRET can greatly enhance the optogenetic performance.

Image



Publications

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Biography: Kanchan Yadav finished her bachelor degree in biotechnology and pursued her master in nanotechnology from Indian Institute of Technology, Roorkee. Later she joined the doctoral program in department of chemistry, National Taiwan University, Taiwan. Her research interest focus on Upconversion nanoparticles (UCNP's) for the medical and health care applications and focusing on Novel two-dimensional (2D) for low detection biosensing in blood and biological fluids.