Abstract

Optogenetics enables precise regulation of cellular functions through the genetic introduction of light-responsive proteins and optical stimulation. Conventional optogenetic approaches predominantly rely on ultraviolet (UV) or visible light, which suffer from limited tissue penetration and high phototoxicity, thereby restricting non-invasive and deep-tissue applications. Upconversion nanoparticles (UCNPs), capable of converting near-infrared (NIR) light into higher-energy UV and visible emission, provide a promising strategy to overcome these limitations by exploiting the superior tissue penetration and low phototoxicity of NIR light.

In addition to microbial opsins that function as light-gated ion channels, G protein-–coupled receptor (GPCR)-type opsins offer an attractive alternative because they activate intracellular signaling cascades via G proteins, enabling signal amplification and highly sensitive cellular responses. Among GPCR-type opsins, bistable opsins are particularly attractive because they can be reversibly switched between active and inactive states by different wavelengths of light. However, their application in combination with UCNPs remains challenging. In particular, for the bistable opsin neuropsin (OPN5), both the activation and inactivation wavelengths overlap with the emission bands of commonly used UV-emitting UCNPs, raising a fundamental question as to whether selective and reproducible optical control is achievable using UCNP-mediated NIR excitation. The objective of this dissertation is to investigate whether UCNP-mediated NIR excitation can enable effective optical control of OPN5 despite this spectral overlap, by systematically examining UCNP design, cell-surface targeting strategies, and optogenetic responses.

In Chapter 2, the formation mechanism and optical properties of $NaYF_4$:Yb,Tm-based UCNPs are investigated, with particular attention to the α–β phase transition, self-focusing growth during Ostwald ripening, and size-dependent luminescence behavior. UV-emitting UCNPs suffer from nonradiative relaxation via lattice phonons and surface defects, particularly affecting high-energy excited states such as the Tm^{3+} $^1D_2$ level. To address this limitation, core–shell UCNP architectures are designed to suppress surface quenching and enhance UV emission, demonstrating that highly crystalline β-$NaYF_4$ UCNPs with appropriate particle size are essential for OPN5-based optogenetic applications.

In Chapter 3, a strategy to selectively target UCNPs to the cell surface of OPN5-expressing cells (OPN5-HEK) is established. UCNPs are rendered water-dispersible by encapsulation with biotinylated phospholipids while retaining stable emission properties and low cytotoxicity. An OPN5 expression construct is designed and constructed to present a FLAG tag on the extracellular domain using a Snorkel system, enabling specific cell-surface targeting. Biotin-functionalized UCNPs are then bound to OPN5-HEK via biotin–avidin interactions, allowing efficient local photon delivery to membrane-localized OPN5.

In Chapter 4, the photoreaction properties of OPN5 are evaluated using Ca^{2+} imaging. Despite the spectral overlap between UCNP emission and both activation and inactivation wavelengths, NIR irradiation induces Ca^{2+} responses when UCNPs are targeted to OPN5, and kinetic analysis reveals distinct response patterns across experimental conditions, suggesting enhanced activation efficiency through spatial localization of UCNPs.

Finally, Chapter 5 summarizes the overall findings and discusses future perspectives for UCNP-based optogenetics. In conclusion, this dissertation demonstrates that bistable opsin signaling can be modulated using UCNP-mediated NIR excitation even in spectrally challenging systems such as OPN5, and suggests a viable approach toward non-invasive optogenetic control of GPCR-type bistable opsins through integrated nanoparticle, opsin, and spatial design.

Keywords: *optogenetics*; *upconversion nanoparticles*; *bistable opsin*; *OPN5*; *near-infrared light*; *Ca^{2+} imaging*