Chemigenetic Tools for Live-Cell Fluorescence Microscopy

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Synthetic fluorophores, in combination with self-labeling protein tags, have become indispensable tools in fluorescence microscopy. Compared to fluorescent proteins, synthetic fluorophores show enhanced brightness, photostability, and are available in the far-red to near-infrared spectral region, which is beneficial for advanced fluorescence microscopy applications including super-resolution microscopy. Moreover, fluorogenic fluorophores, which become fluorescent only upon target binding, are particularly well suited for live-cell applications due to their high signal-to-background ratios. While synthetic chemistry allows fine-tuning of a fluorophore's photophysical properties, the protein environment surrounding the fluorophore binding site also plays a crucial role. Here, we demonstrate how protein engineering of the self-labeling protein HaloTag can be harnessed to develop innovative tools for live-cell fluorescence microscopy.

First, we present a series of engineered HaloTag variants with altered brightness and fluorescence lifetime characteristics that enable multiplexed imaging of up to three distinct targets with in a single spectral channel using fluorescence lifetime imaging microscopy (FLIM). Second, we explore the use of HaloTag beyond its conventional role as a labeling tag by integrating it into functional biosensors. Specifically, we sandwiched a circularly permuted version of HaloTag7 between a phosphoamino acid binding protein and a protein kinase A (PKA) specific peptide to get access to HaloTag-based kinases activity reporters (HaloKARs). Through protein engineering, we optimized the biosensor's performance ultimately reaching a 12-fold change upon PKA stimulation. The best HaloKAR was successfully applied in advanced multiplexing experiments and functional super-resolution microscopy measurements.

Overall, our work underscores the power of combining protein engineering with synthetic fluorophores to create imaging tools with outstanding properties. We expect these HaloTag-based tools to facilitate the widespread use of advanced microscopy techniques.