# Prototype Screening and Optimization of HaloTag-based Chemigenetic Fluorescent Indicators

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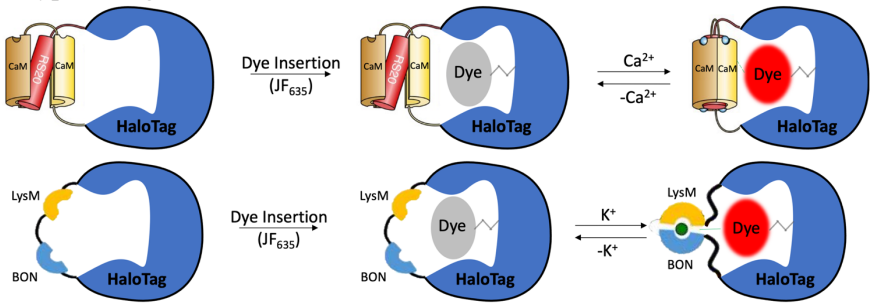
Bioimaging using fluorescent indicators has revolutionized modern biology. Recently, a new class of self-labeling chemigenetic indicators has been developed. In these chemigenetic indicators, a synthetic fluorophore is introduced onto a protein framework consisting of a self-labeling protein and a target-binding domain. The fluorescence intensity is altered due to a conformational change resulting from binding of the target analyte. To date there're only a few representative examples of chemigenetic indicators, the HaloCaMP1 and rHCaMP $Ca^{2+}$ indicators[1,2] and the HASAP1 Voltage indicator[2]. As of yet, this approach hasn't been shown to be broadly applicable to many analytes. Furthermore, optimization and improvement of first-generation indicators are largely unexplored[3].

Here, we explore the chemigenetic indicator's versatility based on the self-labeling HaloTag protein, a fluorogenic rhodamine derivative dye[3], and a protein-based molecular recognition moiety. In principle, this design strategy should enable the versatile design of different indicators by simply changing the sensing domain of the protein framework. So far, we have designed two kinds of chemigenetic indicators based on this framework: a $Ca^{2+}$ indicator and a $K^+$ indicator.

In this work, we inserted the sensing domain (calmodulin-RS20 for the $Ca^{2+}$ indicator, and kbp for the $K^+$ indicator) into different sites on the loop of the HaloTag protein from position 143 to 178. We then treated each protein with a synthetic fluorophore ($JF_{635}$-HTL, which has a chloroalkane linker[3]) and tested the fluorescence change in response to $Ca^{2+}$ and $K^+$, respectively (**Figure**). In total, 32 different insertion sites for each indicator design were tested. We found that inserting the sensing domain onto the loop around position 165-166 of HaloTag gave the highest fluorescence response for the $Ca^{2+}$ indicator (HaloCI), and the position 145-146 gave highest fluorescence response for the $K^+$ indicator (HaloPI). Both showed similar $\lambda_{ex}$ (~658 nm) when labeled with $JF_{635}$-HTL. Both HaloCI and HaloPI showed robust fluorescence intensity change in response to $Ca^{2+}$ and $K^+$ respectively (-94% for 39 μM of $Ca^{2+}$, +88% for 100 mM of $K^+$).

In summary, our results demonstrate the versatility of this chemigenetic indicator design. In future work, we will pursue indicators for additional target molecules, and further optimize the indictor prototypes using directed evolution.

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