A chemi-genetic Ca^{2+} indicator based on a synthetic chelator and a fluorescent protein

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Metal ions (e.g., Ca^{2+}, Na^{+}) are crucial for cell signaling. For example, the Ca^{2+} concentration inside cells controls various processes, such as the release of neurotransmitters and muscle contraction. Fluorescent indicators are widely used for dynamic imaging of biological systems to understand their detailed mechanisms. The main classes of indicators are small molecule-based[1] and protein-based[2] indicators. A small molecule sensor consists of a synthetic fluorophore and a recognition motif, and a protein-based biosensor is made of a fluorescent protein (FP) and an ion-binding protein. Relative to small molecule-based indicators, protein-based indicators are inherently biological compatible and can be expressed with specific localization in cells or tissues. However, since protein-based biosensors rely on the ion-binding proteins, for ions for which suitable binding proteins have not been discovered (e.g., Mg^{2+}, Na^{+}), the development of a biosensor is impossible.

To combine the advantages of synthetic molecules and proteins in a single indicator, we conceived a new indicator design based on integrating a synthetic chelator and a FP. In this work, we set out to construct a Ca^{2+} indicator based on a combination of green FP (GFP), a self-labeling protein (HaloTag) which can covalently bind with a chloroalkane ligand, and a Ca^{2+} chelator BAPTA (1,2-bis(o-aminophenoxy) ethane-*N,N,N,N'*-tetraacetic acid) (**Fig. 1**). After combining ten types of BAPTA ligands and 32 types of GFP-HaloTag proteins, we eventually identified a prototype sensor with a 1.4-fold fluorescence change. We then performed many rounds of rational and random optimization to reach the final variant with a 10-fold fluorescence increase upon binding to Ca^{2+}. We demonstrated that this sensor is applicable to cell-based imaging experiments.

We hope our work serves as the archetype for a new class of chemi-genetic indicator with ion- or molecular-specificities that have not yet been realized with fully protein-based biosensors.

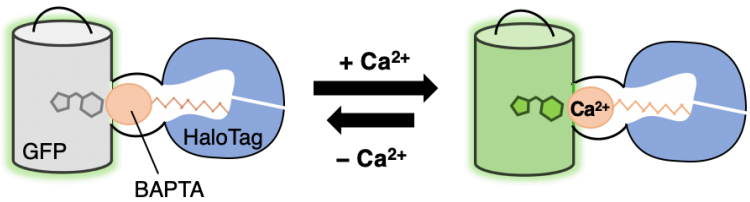

Fig 1. Schematic illustration of the chemi-genetic Ca^{2+} indicator developed in this work.

1) *Curr. Opin. Chem. Biol.* **2008**, *12*, 515. 2) *Nat. Methods*. **2019**, *16*, 649.