Rapid construction of L-lactate biosensor color variants by transposon-mediated insertion

([1]*Department of Chemistry, Graduate School of Science, The University of Tokyo*, [2]*Department of Chemistry, University of Alberta*) ○Fu CHAI,[1] Karl Matthew LIN,[1] Yusuke NASU,[1] Takuya TERAI,[1] Robert E. CAMPBELL[1,2]

Keywords: single fluorescent protein-based biosensor; transposon-mediated insertion; L-lactate biosensor

L-Lactate is increasingly being recognized as playing an important physiological role as an energy currency that is exchanged between cells in animal tissues.[1] With this increasing recognition comes the need for new and improved tools for visualizing L-lactate *in vivo*. A promising class of tools for meeting this need are the single fluorescent protein (FP)-based biosensors, which enable mapping and monitoring of biological activities with high spatiotemporal resolution in a native cellular context (**Figure**).[2] Traditionally, such biosensors have been engineered by rational design followed by protein engineering to improve the allosteric connection between a ligand binding domain and a fused FP. However, the identification of effective connection allostery is the rate-limiting step due to its time-consuming and labor-intensive nature. Recently, a transposon-mediated insertion technique, which constructs a random insertion library containing all possible connections between the two domains, emerged as an attractive technique to interrogate the effective allosteric connection between FP and sensing domain.[3] This study aims to advance the biosensor engineering by a modified transposon-mediated insertion technique using end-optimized transposons to introduce optimal length linkers between the two domains.

In this study, we describe the optimization of three circularly permutated FP color variants for modified transposon-mediated insertion into a binding domain with optimal linker lengths. Application of this method to an L-lactate binding domain (LldR), followed by screening of random insertion libraries, led to a series of L-lactate biosensor prototypes with favorable dynamic range and different colors. This methodology will benefit biological research by accelerating biosensor development and ultimately delivering an improved spectral palette of single-FP based biosensors for multiplex imaging.

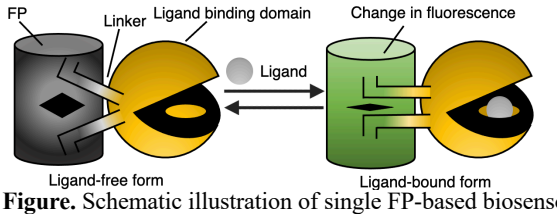

Figure. Schematic illustration of single FP-based biosensor.

1) J. Rabinowitz, S. Enerback, *Nat. Metab.* **2020**, 2, 566. 2) Y. Nasu, Y. Shen, L. Kramer, R. Campbell, *Nat. Chem. Biol.* **2021**, 17, 509. 3) D. Nadler, S. Morgan, A. Flamholz, D. Savage, *Nat. Commun.* **2016**, 7, 12266.