Enhancing the signal response of auto-fluorescent protein-based NO biosensor

([1]*Institute of Advanced Energy, Kyoto University*, [2]*School of Medicine, University of Occupational and Environmental Health,* [3]*Graduate School of Engineering, Kyoto University*)
○ Shunsuke Tajima,[1] Eiji Nakata,[1] Reiko Sakaguchi,[2] Masayuki Saimura,[1] Yasuo Mori,[3] Takashi Morii[1]

Keywords: Auto-Fluorescent Protein; Nitric Oxide; Structural Change; Fluorescent Biosensor; Transient Receptor Potential Canonical 5 (TRPC5) Channel

Nitric oxide (NO) act as the second messenger in cellular signal transduction processes such as cardio vascular, nervous and immne systems.[1] Developing a simple method for sensitive and selective detection of NO is one of the key requirements for studying the functions of NO. Genetically encoded biosensors based on the auto-fluorescent protein (AFP) are useful to explore cellular dynamics because of their easiness for localization and suitability for the long-time imaging. Structural changes of the recognition module induced by the recognition/reaction event with the target are transduced to a conjugated AFP to induce fluorescence signal changes of AFP.[2,3] However, the optimization process for enhancing the signal response of AFP-based biosensor remains to be explored.[2,3]

An EGFP fused putative NO sensing segment of TRPC5 (EGFP-TRPC5) successfully detected a structural change upon disulfide bond formation in the segment as a small change of fluorescence signal.[4] To construct an NO biosensor based on EGFP-TRPC5, a two-step screening method with deletion of amino acid residues in the NO-sensing module from N- and/or C-terminal was applied to enhance the signal response (Fig 1). The deletion of amino acid residues would bring the disulfide bond more proximal to the EGFP chromophore, which is expected to promote an effective transduction of structural changes upon the disulfide bond formation (Fig 1). In the first screening, the structural changes upon disulfide bond formation of 47 mutants were evaluated by RMSD of the backbone of sensing module with *in silico* simulation. Candidates were selected for *in vitro* measurement as the second screening. Further investigations of the mutants *in vitro* and *in vivo* will be discussed.

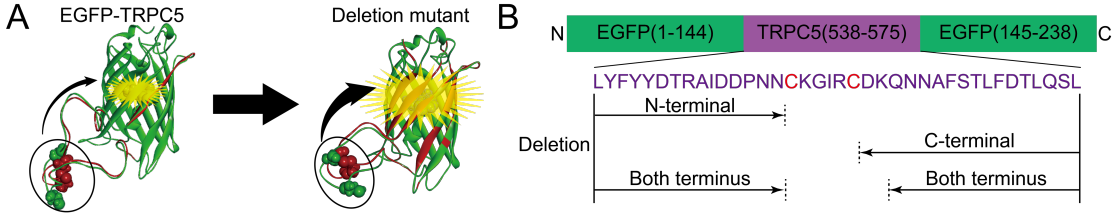

Fig. 1. (A) Deletion of amino acid residues in the NO-sensing module of EGFP-TRPC5 would enhance the signal response upon disulfide bond formation. (B) Illustration of EGFP-TRPC5.

1) DG. Hirst *et al. Methods Mol. Biol.* **2011**, *704*, 1. 2) J. Nakai *et al. Nat. Biotechnol.* **2001**, *19*, 137.
3) M. Jing *et al. Nat. Biotechnol.* **2018**, *36*, 726. 4) S. Tajima *et al. Bioorg. Med. Chem.* **2020**, *28*, 115430.