Optical regulation of protein-protein interactions in living cells using a photochromic dimerizer

Toshiyuki Kowada*

Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Miyagi 980-8577, Japan

E-mail: toshiyuki.kowada.d5@tohoku.ac.jp

Artificial control of protein localization in cells is a powerful tool for elucidating protein functions and for detailed analysis of signaling pathways. One of the representative methods is optogenetics using photoreceptor proteins. However, existing methods for photo-control of protein localization still have issues to be solved in terms of photo-reversibility of protein complex formation and dissociation, dissociation rate of the complex, and relatively large protein domain size that can inhibit the function of the target protein.

To overcome these issues and develop complementary technologies for optogenetics, we developed a photochromic protein dimerizer (pcDH) consisting of a photochromic ligand for a protein tag (eDHFR: *E. coli* dihydrofolate reductase)[1] and a HaloTag ligand (Fig. 1a).[2] By using pcDH, the association and dissociation of eDHFR and HaloTag could be repeatedly controlled in living cells by illumination with violet and green light (Fig. 1b). The half-lives of both association and dissociation were less than 1 second, which is comparable or faster than those for the existing techniques. Furthermore, we applied our system to the optical induction of mitophagy.

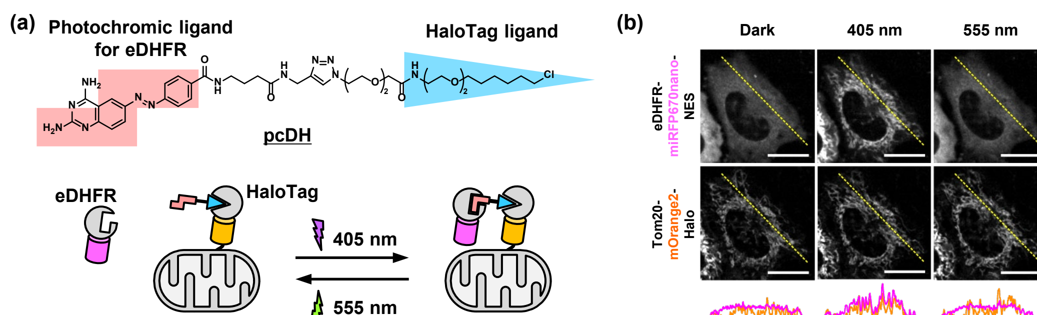

Figure 1. (a) The structure of a photochromic dimerizer, pcDH. (b) Confocal fluorescence images of HeLa cells for photoreversible translocation of cytosolic proteins to the mitochondrial outer membrane.

References

[1] Mashita, T.; Kowada, T.; Takahashi, H.; Matsui, T.; Mizukami, S. *ChemBioChem* **2019**, *20*, 1382–1386.

[2] Mashita, T.; Kowada, T.; Yamamoto, H.; Hamaguchi, S.; Matsui, T.; Mizukami, S. *ChemRxiv* **2023**. DOI: 10.26434/chemrxiv-2022-7vq8s-v2.

Toshiyuki Kowada. Kyoto University, Japan (BS–Ph.D., 2010, Prof. Kouichi Ohe), Osaka University, Japan (Specially appointed researcher and assistant professor, 2010–2014, Prof. Kazuya Kikuchi), Stanford University, USA (Postdoc, 2014–2016, Prof. Jianghong Rao), Tohoku University, Japan (Assistant professor, 2016–present).

[Field of research] Development of fluorescent probes for Zn^{2+} and pH and photo-responsive molecules.