Development of Optochemogenetic Technology for Regulating Intracellular Droplet Formation

([1]*Graduate School of Life Sciences, Tohoku University*, [2]*Institute of Multidisciplinary Research for Advanced Material, Tohoku University,* [3]*Department of Molecular Oncology, Institute for Advanced Medical Sciences, Nippon Medical School*) ○ Muhammad W. Saifudin,[1] Toshiyuki Kowada,[1,2] Hayashi Yamamoto,[3] Shin Mizukami[1,2]

Keywords: Autophagy; Photochromism; Droplet formation; Fluorescence imaging

Biomolecular condensates are intracellular membraneless compartments composed of proteins and/or other biomolecules, formed through multivalent weak interactions to exhibit liquid-liquid phase separation. Because biomolecular condensates are associated with various diseases, cells regulate their quantity through a selective autophagy process called fluidophagy. Autophagosome formation in fluidophagy involves the recognition and sequestration of droplets by the isolation membrane. Notably, only droplets with small size and low surface tension can be fully sequestered.[1] However, the detailed mechanism of autophagosome formation in fluidophagy remains unclear, highlighting the need for a method to manipulate droplet formation and properties, such as droplet size and fluidity, in living cells.

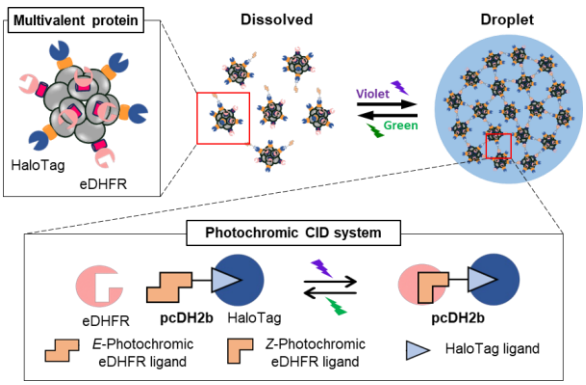

Fig. 1. Strategy for regulating droplet formation.

Recently, we developed a photochromic CID (chemically induced dimerization) system capable of photoreversibly controlling the dimerization of two tag proteins, *E. coli* dihydrofolate reductase (eDHFR) and HaloTag, using a photochromic dimerizer, **pcDH**.[2] We also improved this photochromic CID system by combining a newly developed photochromic dimerizer, **pcDH2b**, with a tag protein mutant, thereby reducing background association. Based on this improved photochromic CID system with a multivalent protein (Fig. 1), we developed a novel technology for optically controlling intracellular droplet formation. In this presentation, we will discuss the results regarding optical control of droplet formation and dissolution, including optical control at the sub-cellular region and droplet size regulation.

Reference:
1) J. Agudo-Canalejo *et al.*, *Nature* **591**, 142 (2021). 2) T. Mashita *et al.*, *Nat. Chem. Biol.* **20**, 1461 (2024).