Development of photocatalytic-proximity protein labeling for profiling protein-protein interaction in intracellular microenvironments

([1]*Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology,* [2]*School of Life Science and Engineering, Tokyo Institute of Technology,* [3]*Frontier Research Institute for Interdisciplinary Sciences, Tohoku University*) ○Michihiko Tsushima,[1,2] Shinichi Sato,[3] Hiroyuki Nakamura[1]

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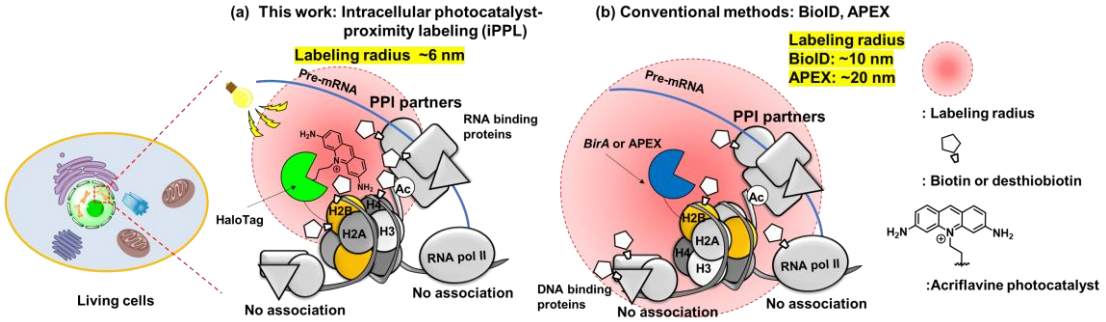
Protein–protein interactions (PPIs) are a key component of molecular interaction networks, and thus, techniques for analyzing PPIs can yield key elucidations of biological pathways. Although proximity labeling techniques utilizing genetically expressed proteins of interest (POIs) fused to enzymes such as *BirA* (BioID)[1] and engineered ascorbate peroxidase (APEX)[2] were useful for clarification of protein dynamics and localization in living cells, it is difficult to apply them to focused PPIs profiling due to their large labeling radius (BioID: ~10 nm, APEX: ~20 nm).

In this study, we developed intracellular photocatalytic-proximity labeling (iPPL). Acriflavine was found to be an efficient cell-membrane-permeable photocatalyst for introduction into genetically fused HaloTag for photocatalyst-mediated labeling with 1-methyl-4-arylurazole (MAUra)[3] within a few-nanometer labeling radius. We demonstrated the photocatalyst-proximity labeling of H2B as a POI with MAUra in the intracellular environment of HaloTag-H2B expressed HEK293FT cells. Endogenous histone components (H2A, H2B, H3, and H4) and RNA-binding proteins that directly interact with histone were selectively labeled using the iPPL approach, revealing that iPPL has approximately 6 nm labeling radius which is smaller than those of BioID and APEX.



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